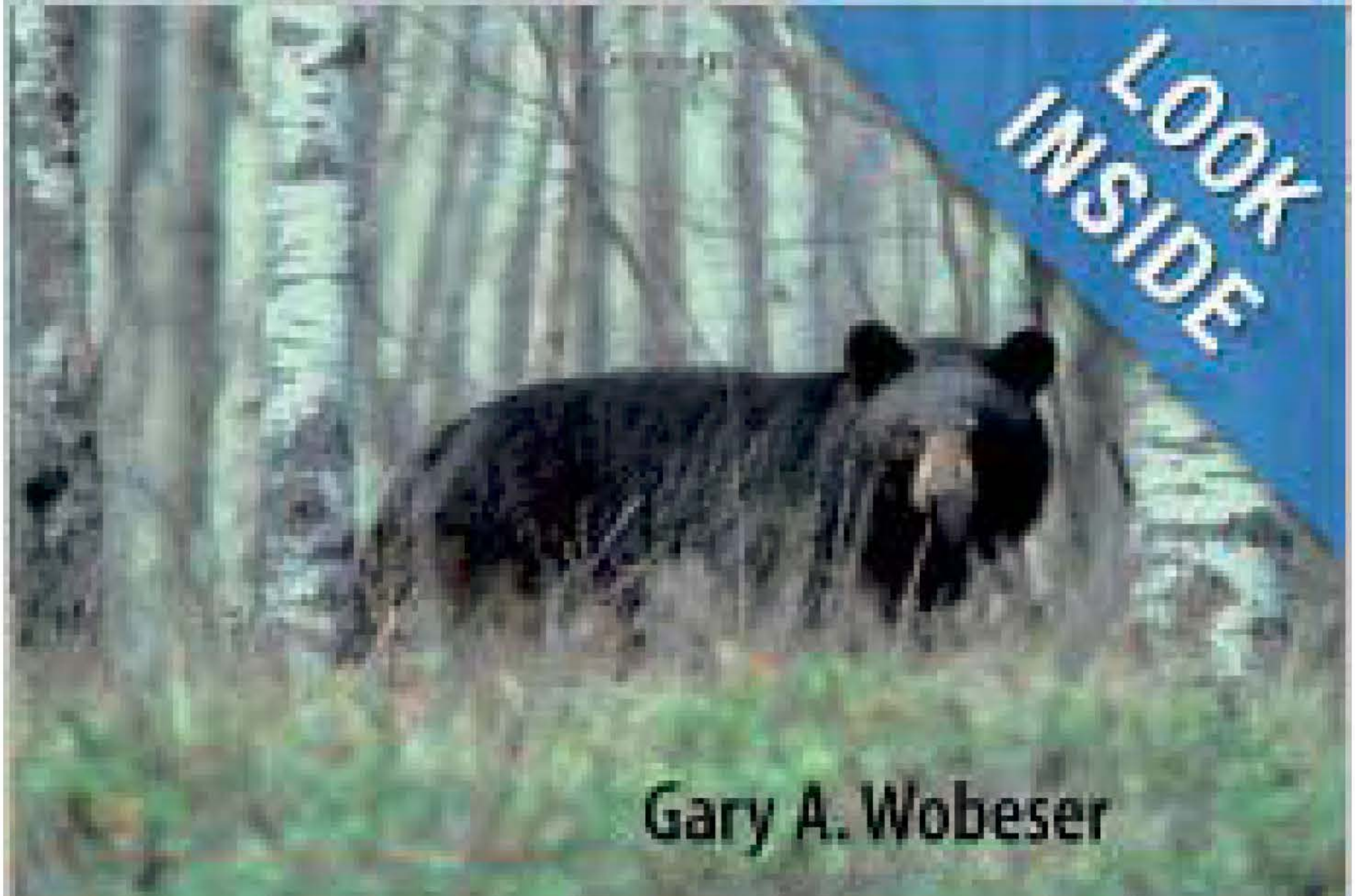

Gary A. Wobeser

Disease in Wild Animals

Investigation and Management

Second Edition

Springer

Disease in Wild Animals: Investigation and Management

2nd Edition

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Disease in Wild Animals
Investigation and Management

2nd Edition

With 17 Figures

Springer

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Preface

This book arose out of teaching graduate and undergraduate classes in wildlife diseases. It, in some ways, chronicles my involvement in the investigation and diagnosis of diseases in free-ranging wildlife, primarily in western and northern Canada, since the 1960s. It also, perhaps, reflects the development of wildlife disease study as a discipline. Much of the earlier work in this field was purely descriptive, documenting the occurrence of various diseases in wild animals. I have chosen to retain references to some older and obscure information in this second edition because this body of work provides the foundation for a more analytical approach. The literature on health problems in free-ranging animals is expanding rapidly. I am gratified that the theoretical and quantitative aspects of wildlife disease are receiving more attention than in the past, and that role of disease as a factor in population biology is being analyzed. My hope for the first edition of this book was that it would serve as an overview of the study of disease in wild animals and of methods that might be used to manage health problem. It was, and is, not intended to be a how-to book or an encyclopedic reference to the literature on disease; rather it is intended as a seed crystal around which the reader can build. The inquiries I have received about a second edition suggest that it has been useful. The field of wildlife diseases is an interface area between medicine and applied biology. During the past half-century, medical science has become preoccupied with technology and with dissecting disease phenomena at the molecular level in the laboratory. This has resulted in marvelous tools for the study of disease agents. However, study of disease in whole animals and of the population biology of disease became unfashionable, even though such knowledge is essential if the results of high-tech research are to be applied. In contrast, wildlife biology is concerned with populations and, to the wildlife manager, disease is important only when it has an impact on the population. Some basic concepts of epidemiology, such as mortality rate and survival rate of a population, are used more frequently by the average biologist than by the average health practitioner. Medical scientists don't think of disease in terms of fitness, trade-offs or compensation, but these concepts are fundamental to the ecologist. The role of the "wildlife disease specialist" is to use the tools of biomedical science within an ecological framework to understand how and why disease occurs in free-living populations and when and how it might be managed.

I thank my wife Amy Grace for her patience and support; my colleagues in the Department of Veterinary Pathology, Western College of Veterinary Medicine, and the Canadian Cooperative Wildlife Health Centre for allowing me to pursue my interests; the students who have tolerated my enthusiasm and served as a sounding board for notions; and the many wildlife biologists I have worked with over the years who have helped me to keep the importance of disease in perspective.

Saskatoon, December 2006 *Gary A. Wobeser*

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Section I
Introduction

"*Up to the present time it has been customary to believe that wild animals possess a high standard of health, which is rigidly maintained by the action of natural selection, and which serves as the general, though unattainable, ideal of bodily health for a highly diseased human civilization. This belief is partly true and partly false.*"

(Elton 1931)

1 Disease and epizootiology—basic principles

> "*Typically, diseases of wildlife have been investigated by performing pathological examinations on carcasses that are found incidentally, or producing lists of parasites identified in small samples of host species. There have been few attempts to assess the impact of a disease on the host population rather than the individual, or to describe the distribution of the disease agent in a manner sufficient to understand its epidemiology.*"
>
> (Gulland 1995)

1.1 Disease and diseases

The concept of disease is surprisingly difficult to define in terms that are sufficiently broad for application to the wide range of conditions that occur in free-ranging wild animals, and that are still sufficiently narrow to separate disease from other factors, such as predation and food supply, that affect wildlife negatively. Disease might be defined as any departure from health, but this leads to a circular discussion of the meaning of health and normality. Disease in wild animals is often considered only in terms of death or obvious physical disability, probably because these are readily identified parameters. However, the effect of disease on wild populations may be much greater than is evident by simply counting the dead or maimed, even if it were possible to do so accurately. The impact of DDT and certain other chlorinated hydrocarbon insecticides on some raptorial and piscivorous birds provides an excellent illustration of this fact. These compounds have low direct toxicity and rarely result in the death of birds or in obvious clinical signs of intoxication, yet they had profound population effects through decreased recruitment as a result of increased egg breakage.

Disease conditions should not be dismissed as inconsequential simply because they occur commonly, nor should one assume that a disease condition or parasite has a major effect on the host simply because it is conspicuous. Infection with parasites of various types is ubiquitous in wild animals and reference is often made to *normal* parasite burdens, the inference being that parasites at this level have little or no impact on the animal. However, the actual effect of these parasites on the host is largely unknown. "*Although most infectious agents do not result in obvious disease, the host must pay a 'price' for harboring parasites that live, grow, and reproduce at the expense of the host*" (Yuill 1987). In domestic livestock, this price can be measured in

terms of decreased efficiency of production and, even in livestock, the true extent of the cost is often not recognized until the parasite or disease has been eliminated. This type of assessment is seldom possible in free-ranging animals but observations from semi-free-ranging animals, of the same species, such as those on game-farms, provide some indication of the cost of parasitism. For example, Szokolay and Rehbinder (1984) reported a 20% increase in the growth rate of fallow deer after gastro-intestinal parasites, of the type common in wild deer, were partially controlled through the use of anthelmintics. Perhaps even more pertinent is the finding that treatment with anthelmintics resulted in an "*almost 100% increase in body weight in the fawns*" and increased antler growth in males among free-ranging roe deer (Duwel 1987). Special techniques may be required to assess the cost of a disease. For example infection with avian malaria (*Plasmodium pediocetii*), while not causing obvious illness in male sage grouse, had a significant effect on breeding that was only detected in detailed behavioral studies. Infected males attended the lek less regularly, copulated less frequently, and bred later in the season, with less "*fit*" females, than did non-infected males (Johnson and Boyce 1991). Female sage grouse selected against mating with males that had hemorrhagic spots on their air sacs of the type produced by lice (Spurrier et al. 1991). We currently do not have sufficient techniques for measuring effects such as subtle alterations in behavior or diminished intelligence that have been documented to occur in parasitized humans (Levav et al. 1995; Flegr et al. 2003).

The effect of disease may only be evident under certain conditions. For instance, infection with blow fly larvae (*Protocalliphora* sp.) had no effect on the weight, size, or age at fledging of young sage thrashers; however, parasitized birds had a higher mortality rate than unparasitized fledglings during a period of wet, cold weather, suggesting an interaction between parasitism and other stressors (Howe 1992). Similarly, Murray et al. (1997) found a synergy between intestinal parasites and nutrition in snowshoe hares when food was limited. It also is important to examine the correct portion of the population in evaluating the effect of disease. Iason and Boag (1988) failed to find any correlation between intensity of infection with an intestinal parasite and body condition or fecundity of adult mountain hares but suggested that it would be very important to determine the effect of the parasite on growth and survival of young hares before concluding that it was harmless. The members of any population are not homogenous and a small proportion of the population may bear the brunt of a disease. This is most clearly established for infections by larger parasites in which "*most hosts have very low parasite burdens and a few hosts have very high burdens*" (Shaw et al. 1998) but the same principle likely applies to many other diseases in which underprivileged individuals in the population are affected disproportionately. It may be very difficult to detect or measure the impact of disease in these situations because severe effects on a small number of animals may have relatively little effect on measures of central tendency such as the average rate of growth or the median body condition.

1.2 A definition of disease

The definition of disease that will by used in this book is that the term includes "*any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factors*" (Wobeser 1997). No distinction will be made between infectious and non-infectious causes of disease because the basic principles of investigation and control are similar for both. However, the term **risk factor**, rather than **causative agent**, will be used when referring to some non-infectious diseases.

Within this broad definition of disease, groups of affected animals with similar features are classified into categories or are said to be affected by a particular *disease* that is identified by a specific name. There is no consistent pattern or rationale for naming diseases, so the current situation represents a hodge-podge of styles:

Name of disease	Derivation of name
Tyzzer's disease	Discoverer (E.E. Tyzzer)
Tularemia	Locale of first description (Tulare County, California)
Bluetongue	Clinical feature
Enzootic ataxia	Clinical and epizootiological features
Avian vacuolar myelinopathy	Pathological feature
Aspergillosis	Causative agent (*Aspergillus* spp.)

In many cases, the name applied to a disease reflects the current understanding of its nature and this name is open to change as the cause or nature of the disease is elucidated. Categories or diseases may be subdivided when it is discovered that several causes produce similar features. For instance, the disease tularemia is now known to be caused by four closely related bacterial species in the genus *Francisella* and types A and B tularemia are recognized. In general, the tendency with time is to define the characteristics of a disease more precisely, and to indicate the causation in the name. For example, a common disease of domestic cattle has gone through a progression of names from red nose, to infectious bovine rhinotracheitis, to bovine herpesvirus I infection. Unfortunately, several names may be applied to a single disease simultaneously, resulting in unnecessary confusion. Thus, a single disease of waterfowl caused by one virus is referred to as duck plague, duck virus enteritis, duck viral enteritis, and anatid herpesvirus infection.

1.3 Disease causation

The study and understanding of the cause and nature of disease have undergone a gradual evolution. Prior to discovery of the identity of specific infectious agents, there was considerable controversy between the alternate hypotheses of the *contagium vivum* (or living contagion theory) and the *miasma* or (bad air theory). The discovery of microbial pathogens silenced this controversy for a period and, at the turn of the past century, both human and veterinary medicine were concerned primarily with identification of specific agents responsible for acute infectious diseases. A set of rules (Koch's postulates) developed for establishing cause-and-effect relationships between infectious agents and disease were generally accepted and widely applied. These stated that the agent:

- must be shown to be present in every case of disease through its isolation in pure culture;
- must not be found in cases of other diseases;
- must be capable of reproducing the disease in experimental animals;
- must be recovered from the host in which experimental disease was produced.

These laboratory-based criteria for judging causal relationships to disease were valuable in defining many diseases of simple etiology, and are still useful in the study of certain diseases such as rabies. However, this one agent-one disease model is not adequate for either the study of many diseases or for the explanation of how most diseases behave in nature. Koch's postulates are particularly inappropriate for many non-infectious diseases, for diseases caused by mixed infections, for diseases in which the predisposing factors are important, for diseases with a carrier state, for diseases caused by opportunistic agents that may or may not cause disease when present, and for many chronic diseases in which the inciting cause has disappeared before the clinical disease becomes evident. Hanson (1969) eloquently outlined the deficiencies of these postulates for the study of wildlife diseases in a presentation entitled "*Koch is dead*" to the Wildlife Disease Association annual meeting.

A more holistic view is necessary for the understanding of most diseases. Jekel et al (1996) proposed three categories that are useful for considering potential agents or factors as the cause of disease:

- If a **sufficient cause** is present the disease will always occur;
- If a **necessary cause** is absent the disease cannot occur;
- A **risk factor** is a characteristic that, if present and active, increases the probability of the disease occurring.

Multiple features of each of the **agent**, the **host** and the **environment** in which the disease is occurring must be considered in evaluating disease causation

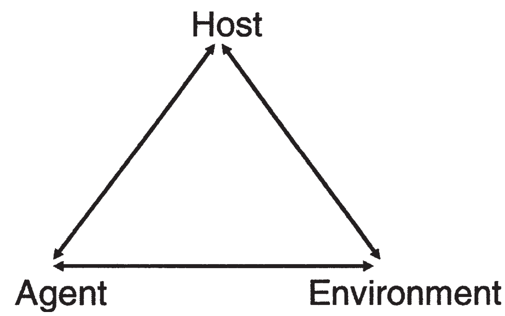

Fig. 1.1 This schematic often is used to symbolize the interactions among agent, host, and other environmental factors that govern the occurrence of disease

(Fig. 1.1). (Consideration of environmental factors recalls the miasma theory of prior times). Even when dealing with a disease caused by a single agent, each of the three major components has a variety of determinants, any of which may influence whether or not overt disease will occur. This simple table presents only a few such variables:

AGENT	HOST	ENVIRONMENT
Strain	Species	Climate
Dose	Genotype	Weather
Route of exposure	Age	Altitude
Duration of exposure	Sex	Other species
	Nutritional status	Population density
	Reproductive status	Air and water quality
	Past exposure	Soil
	Concurrent disease	Human activity
	Immunocompetence	
	Food habits	
	Behavior	

For many diseases, even this agent-host-environment approach may be inadequate and it is more appropriate to consider disease in terms of a **web of causation** in which many factors interact to result in disease. It often is difficult in these situations to classify a factor as being distinctly a feature of the agent, the host, or the environment. Any single factor may be a necessary component but may not be sufficient, in and of itself, to produce disease without the presence of co-factors. The 'lungworm-pneumonia complex' of bighorn sheep (Forrester 1971) provides an excellent example of this type of situation. A wide variety of infectious agents including parasitic nematodes (*Protostrongylus* spp., and less commonly *Muellerius* sp. lungworms), bacteria (*Pasteurella* spp., *Mannheimia haemolytica*, *Arcanobacterium pyogenes*, *Streptococcus* sp., *Staphylococcus* sp., *Neisseria* sp., *Chlamydophila psittaci*, *Mycoplasma* sp.) and viruses (parainfluenza 3 virus, respiratory syncytial virus, bluetongue virus) have been recovered

from sheep dying during outbreaks of pneumonia. It has not been possible to fulfill Koch's postulates completely with any of these agents. Some of the agents are present in healthy as well as in diseased sheep and others have been present in some outbreaks and but not in others, and experimental infection with individual agents either does not result in disease or produces disease that is dissimilar to the natural condition. Many of the agents have been described as predisposing, contributing or opportunistic factors, and none has been identified as **the** cause of the disease. In addition to the infectious agents, many environmental and host risk factors also have been suggested to contribute to the occurrence of this disease. These include overcrowding, interspecific and intraspecific competition, human harassment, poor range quality, contact with domestic livestock, deficiency of trace minerals, above normal rainfall, and inclement weather. Each of these factors is, in turn, influenced by many other factors, so that one could construct a very complex web of causation (Fig. 1.2). Many of the associations in this web are unproven, and will be difficult to prove without experimental manipulation. Even within such a web, it is very tempting to search for a primary cause, and the one agent-one disease concept is still prevalent both among the public and many professionals. (*Pasteurella* spp. and *Mannheimia haemolytica* are the current front-runners among agents considered important in pneumonia in wild sheep). To further complicate the matter, disease complexes such as this often are found, on dissection, to consist of a number of similar but distinct disease entities, each with its own web of causation. This is probably true of pneumonia in sheep in

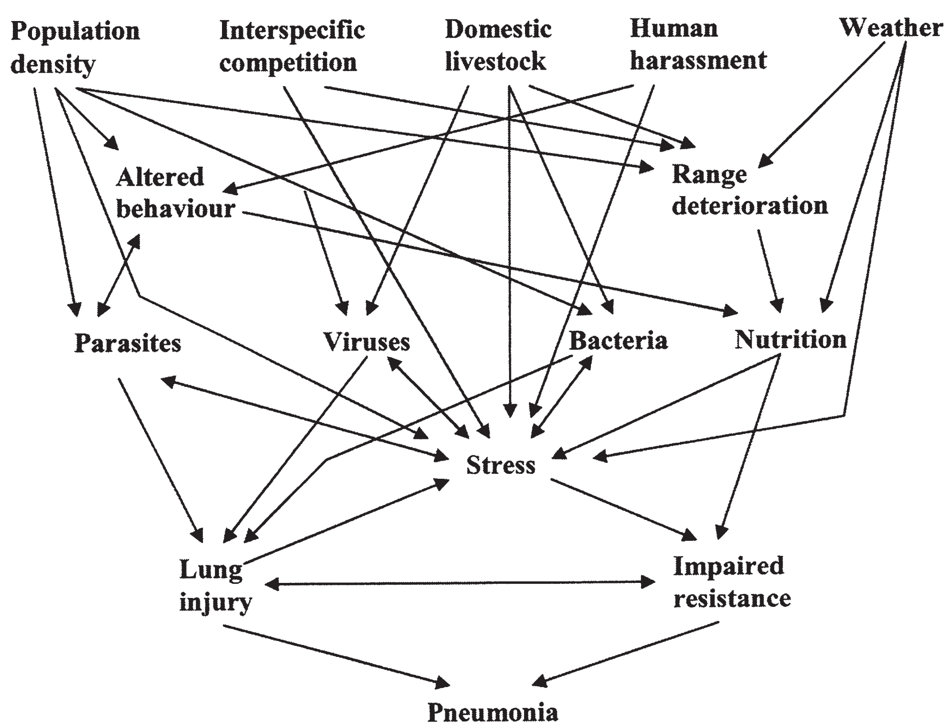

Fig. 1.2 Schematic diagram to illustrate the interrelatedness of various factors that may be associated with, and form a web of causation for, the 'lungworm-pneumonia complex' of wild mountain sheep. Many of these associations are speculative, and the list of factors is likely far from complete

which there appear to be different combinations of viruses, bacteria and other factors in each geographic location with effects on sheep ranging from inapparent infection, through mortality of lambs, to 'all-age' die-offs.

A set of criteria for establishing causation, adapted from Kelsey et al. (1986), reflects the multifactorial nature of most diseases:

1. The hypothesized cause should be distributed in the population in the same manner as the disease.
2. Occurrence of disease should be significantly greater in those exposed to the hypothesized cause than in those not so exposed.
3. Exposure to the hypothesized cause should be more frequent among those with disease than in those without disease, when all other risk factors are constant.
4. Temporally, disease should follow exposure to the hypothesized cause.
5. The greater the dose or length of exposure to the hypothesized cause, the greater the likelihood of occurrence of disease.
6. For some diseases, a spectrum of host responses along a biologic gradient from mild to severe should follow exposure to the hypothesized cause.
7. The association between the hypothesized cause and disease should be evident in various populations studied by different methods.
8. Other explanations for the association should be eliminated.
9. Elimination or modification of exposure to the hypothesized cause should decrease occurrence of the disease.
10. Prevention or modification of the host's response on exposure to the hypothesized cause, e.g., through immunization, should decrease or eliminate disease.
11. Disease should occur more frequently in animals exposed experimentally in an appropriate manner to the hypothesized cause than in control animals not so exposed.
12. All relationships and findings should make biologic sense.

Recognition of the complex interrelationship among various factors allows formulation of hypotheses that can be examined and tested, and points in the web can be identified where control measures might be applied. Jekel et al. (1996) suggested that there are three basic steps in the determination of cause and effect (after putative causes have been identified): (i) investigation of statistical associations between cause and effect, (ii) investigation of the temporal association, and (iii) elimination of all known alternative explanations. These will be explored further in later chapters.

1.4 Disease investigation

The basic reasons for studying any disease are to determine its nature and cause, assess its significance (i.e., to determine the effect of disease on individuals and on the population), and identify methods to prevent, control, or

reduce the disease or its effects. Other reasons for studying disease conditions in wild animals might include curiosity about disease as a biological phenomenon, concern that wild animals have a role in diseases of humans and domestic animals, use of wild animals as monitors or indicators of undesirable changes in the environment, public concern about conditions such as parasites in game animals and highly visible die-offs, concern about the impact of disease on the wild population, and the use of disease to manage pest or problem wildlife.

Disease may be approached through either the study of its course and effects in the individual, or by studying the occurrence, distribution, and effects of the condition in a group or population. These two types of study are often termed **clinical studies** and **epizootiology**, respectively. This book deals primarily with disease in populations but information from clinical studies is required for diagnosing and defining individual diseases. Clinical studies often are required to confirm hypotheses about associations and cause-and-effect relationships.

1.5 Basic epizootiological terms

The words epidemiology and epizootiology often are used interchangeably in discussion of disease in animals. Epidemiology is defined as the study of occurrence of disease in populations and is derived from *epi* = on or upon + *demos* = the populace, and probably dates to the great plagues or epidemics inflicted upon the human populace. Epizootiology has a similar meaning with reference to animals, and will be used in this book. Epizootiology is a quantitative science. The basic methods involve description and characterization of groups of individuals so that quantitative comparisons can be made among groups and so that associations among various factors can be measured. This may involve observation and documentation of naturally-occurring events, such as determining the relative rate of mortality of different age groups during a die-off (**observational epizootiology**), or of studying the effect of some manipulation on the occurrence of a disease (**experimental epizootiology**). A study of the efficacy of a vaccination program for control of rabies in foxes would be an example of the latter type. The objective in epizootiological studies is to collect numerical information that can be applied to the solution of one of the three basic problems of causation, significance, or management.

Description and characterization of disease is done through the use of terms that have a specific and restricted meaning. Unfortunately, many of these terms often are misused. Because a word means what one says it means, some of the terms used most commonly will be discussed here to reduce confusion later.

The general pattern of a disease within a population is characterized by the number of cases that occur over a given time period, relative to the number of cases that would be expected or that would occur normally during that

period. An **enzootic** disease is one that occurs in a population at a regular, predictable, or expected rate. An **epizootic** disease occurs when a disease appears at a time or place where it does not normally occur, or with a frequency substantially greater than that expected for the time period. Thus, an epizootic is said to occur when there is an increase in the number of cases over past experience for a specific population, place and time.

The less precise descriptive terms **outbreak** and **die-off,** which refer to a large number of cases occurring within a short period of time, are not necessarily synonymous with epizootic. For example, sudden explosive outbreaks of botulism occur annually with predictable regularity among waterfowl on some wetlands. Because it occurs on a regular basis, botulism is, by definition, an enzootic disease in these wetlands. Similarly, all males in the population of the Australian dasyurid marsupial *Antechinus stuartii* die abruptly each year (Barker et al. 1978) but this must be regarded as an enzootic event. In contrast, even a single case of a disease may represent an epizootic, if it occurs at a time or place where that disease does not occur normally. Thus, a solitary case of foot-and-mouth disease in a deer, anywhere in North America, certainly would be treated as an epizootic! A single disease may occur in different patterns at different locations, e.g., hemorrhagic disease, a viral disease of deer, is enzootic in white-tailed deer in the southeastern United States but occurs as isolated sporadic epizootics in more northern areas.

Classification of a disease occurrence as either an epizootic or an enzootic event is dependent upon knowledge of its prior occurrence in an area and the classification may change as any new information is gathered. Avian cholera was considered an epizootic disease when it was first discovered among wild geese in Saskatchewan in 1977; however, we know now that the disease occurs at a similar rate each spring within this population and, thus, its status has changed to that of an enzootic disease in this area. Similarly, West Nile virus infection in North America appears to be in the process of changing from an epizootic to an enzootic disease as it becomes established. Packer et al. (1999) used serologic data collected over a 30-year period to classify viral diseases of lions in the Serengeti. Two viruses (feline herpesvirus and feline immunodeficiency virus) occurred enzootically, while four other viruses occurred as discrete epizootics. The terms epizootic and enzootic are based on the frequency of occurrence of cases and not on the severity or duration of the clinical disease in individual animals. Thus, rabies is an enzootic disease in some areas although many animals may be affected and the disease has a short, fatal clinical course. Conversely, a mild, chronic disease may occur in epizootic form.

The most basic quantitative measurement that can be made of a disease is a count of affected individuals. However, such counts may have little value unless they can be put into context. For instance, a count of five striped skunks with rabies has relatively little meaning, except to indicate that rabies is present in the area, unless it can be seen in relation to the group or population from which the animals originated. Finding five rabid skunks would

have much different significance if the animals were found in a sample of ten (i.e., 50% of the sample were affected), than if the five rabid skunks were found in a sample of 1,000 skunks, in which case only 0.5% were affected. A major concern in epizootiology is the identification of suitable population denominators that can be used to convert **counts** into **proportions**. While simple counts are of limited value for making comparisons, proportions can be used in many ways to describe and compare groups. Unfortunately it is often difficult (if not impossible) to identify or count suitable population denominators in wild animals and many studies have resulted only in 'dangling numerators' that are of limited use. If we return to the example of the rabid skunks, the usefulness of proportions versus counts should be obvious. By using proportions, comparisons can be made between areas, years, or age groups:

	County A	County B
Number of rabid skunks	5	5
Number of skunks examined	100	880
	1986	1987
Number of rabid skunks	5	5
Number of skunks examined	10	650
	< 6 months	> 6 months
Number of rabid skunks	5	5
Number of skunks examined	100	1,000

In each of these examples, the raw count of diseased animals was identical in the two subsets of information but the proportion of infected animals was markedly different. Even simple comparisons of this type may provide valuable insights to the disease.

Certain specific proportions or rates are used frequently in epizootiology. The most commonly used of these, **prevalence** and **incidence**, often are misunderstood and misused. **Prevalence describes the frequency of occurrence of disease within a group at a specific point in time,** i.e., prevalence = the number of animals with disease at a specific time divided by the total number of animals in the group at the time. Theoretically, the point in time should be an instantaneous snapshot or cross section of the group. In practice, measurements are usually made over a short period of time, such as one or a few days. Thus, the apparent prevalence of ringworm, a fungal infection, among mule deer killed by hunters in southern Saskatchewan during period of a few days in 1985 was 1.82% (9/494) (Wobeser, unpublished data). (The term "apparent prevalence" is used here because it is unlikely that all of the deer that may have been infected would have been detected by simple visual exam-

ination of the dead animals, thus, the true prevalence of infection was likely higher). Estimating the true prevalence may be important in some situations, such as when trying to assess progress toward eradication of a disease, e.g., see O'Brien et al. (2004). Although the point of measurement for prevalence is usually a period in time, it also may be an event that happens to different individuals at different times. For example, it is correct to calculate the prevalence of congenital anomalies in duck embryos examined on the 20th day of incubation or the prevalence of antibodies to a particular agent among 4-month-old fawns.

Incidence describes the rate of development of a disease within a group during a specified period of time, i.e., incidence = the number of animals developing disease per unit time divided by the number of animals at risk in the group. Incidence refers only to **new** cases that develop during the time period. Animals that had the disease or that were immune to the disease at the beginning of the period are excluded from the denominator, because they were not at risk of developing the disease. Incidence rates are used less frequently than prevalence rates in studies of wildlife, because of the difficulty in following individual animals over a period of time. The most common method used to measure incidence is to examine animals at the start of a time period and then to examine them again at some later date. The incidence rate is calculated from the number of new cases that have developed during the interval. An example of this type of study is the use of sentinel chicken flocks to monitor the amount of arthropod-borne viruses, such as western equine encephalomyelitis virus, occurring in an area. Chickens known to be free of the disease and that do not have antibodies (and, hence, are at risk) are placed in the environment and then monitored at regular intervals for infection or the occurrence of antibodies. The incidence rate (based on the number of new cases detected during the time period) provides an index of the amount of virus activity in the area during the period. This rate can be compared with the incidence rate in other years, and the information can be used to predict the likelihood of an epizootic. A similar technique has been used to measure the incidence of West Nile virus in nestling wild birds. The information can be used to assess the need for management measures, such as vaccination of horses, and measures to reduce human exposure to mosquitoes.

Incidence rates also can be calculated in other ways. For example, the incidence of exposure to a disease in deer during the first 6 months of life could be calculated by measuring antibodies to the agent in blood collected from fawns killed by hunters in the autumn. This approach includes certain verifiable assumptions including that:

- all deer were uninfected and susceptible at birth;
- exposure did not result in the death of deer;
- antibodies are the result of active exposure;
- all infected animals develop antibodies;
- antibody titres persist at measurable levels for at least 6 months.

The rate calculated from such a study would probably underestimate the true rate of exposure because recently exposed animals may not have had an opportunity to develop antibodies and some animals may fail to develop antibodies, even though they were exposed. Despite these deficiencies, the information could be used to make comparisons with similar data from other areas or other years.

In many instances in wildlife disease work, the rates measured are neither true prevalence nor true incidence rates. For example, the proportion of caribou found to be infected with brucellosis among a sample collected throughout an entire year is comprised of the prevalence of the disease at the start of the year, plus the incidence (new cases) throughout the year. In such instances, it is likely more appropriate to report a simple proportion, such as the proportion of infected caribou among the sample collected during the year, rather than using either prevalence or incidence. Qualified terms such as period prevalence rate in which the time period is specified also might be used.

Other rates used less frequently than prevalence and incidence are the morbidity, mortality, and case fatality rates. The morbidity rate is the number sick and mortality rate is the number dying during a unit of time divided by the number in the group during that time. These are analogous to an incidence rate but measure illness and death rather than occurrence. The case fatality rate is the number of individuals dying of a disease divided by the total number with the disease, and can be used as a measure of the severity of the disease. Any of these rates may be applied to subgroups within a population, such as subgroups based on sex or age. For example, the age-specific apparent prevalence of ringworm in the mule deer mentioned earlier was:

Age class (years)	Prevalence of ringworm
0.5	0.6% (1/181)
1.5	2.1% (2/94)
>2.5	2.7% (6/219)

The prevalence of two different factors within a group of animals may be determined and this information may be used to measure the strength of association between the factors. For example, assume that a group of caribou was examined for the presence of carpal bursitis (inflammation of a bursa on the fore-legs) and for antibodies to the bacterium *Brucella suis* biovar 4, which is thought to be associated with bursitis in caribou. The data below show how the relationship of one variable to another can be examined in a simple 2×2 contingency table. In this example, a sample of 400 caribou was examined and divided into four groups:

	Carpal bursitis		
	Present	Absent	Total
Antibodies			
Present	20 (a)	28 (b)	48 (a + b)
Absent	2 (c)	350 (d)	352 (c + d)
Total	22 (a + c)	378 (b + d)	400 (a + b + c + d)

Twenty animals had both bursitis and antibodies, while 350 had neither antibodies nor bursitis. The prevalence of bursitis was 5.5% (22/400) and the prevalence of antibodies to *Brucella* was 12% (48/400). The data can be used to examine the association between bursitis and *Brucella* infection; the first step in investigating a causal relationship suggested by Jekel et al. (1996). The prevalence of bursitis in those animals previously exposed to *Brucella*, as indicated by the presence of antibodies, was 41.7% (20/48), whereas the prevalence of bursitis in those without antibodies was only 0.6% (2/352). One way of measuring the relative strength of association between two factors is through calculation of the **odds ratio** (ad/bc), which is the ratio of disease occurrence between the two groups. If there is no association between the factors, the odds ratio would be 1. In this case the odds ratio is 125, which indicates a strong association between bursitis and exposure to *Brucella*. This does not prove a causal relationship between brucellosis and bursitis but it does indicate an association worth exploring further. This and other methods for examining the strength of the association between a factor and a disease will be discussed further in subsequent chapters.

Many features of disease, such as growth depression, antibody titre, number of parasites, and thickness of eggshells, occur on a quantitative or continuous scale, rather than on a qualitative or yes-no relationship. Such attributes are described in terms of distribution, measures of central tendency (mean, median), and of dispersion (range, variance, standard deviation). Comparison among groups in these instances is by standard statistical methods. (Because of the homogeneity in relative susceptibility within a population, it may be important to consider both the variance as well as measures of central tendency to detect effects on small segments of the population). The severity of expression of disease is often proportional to the level of the causative agent present and, as noted earlier, observation of a dose:effect gradient is generally considered to be evidence for a causal relationship. For example, the thinning of eggshells caused by certain pesticides has been shown to be directly proportional to the concentration of the chemicals within the egg in several species of bird (Blus et al. 1972; Ohlendorf et al. 1978). This type of relationship can be measured by standard methods for determining correlation and regression among the variables. This system of investigation can be extended to the simultaneous collection of information

on a large number of agent, host and environmental variables, and analysis of the resulting data by multi-variate analysis. In this way, the strength of association among many factors to the disease can be measured. Carey et al. (1980) provide an excellent example of this type of study in their characterization of the landscape epizootiology of Colorado tick fever.

Highly sophisticated techniques are available for the collection and analysis of epizootiologic data; however, the investigator must always be concerned about: (i) how representative the samples are of the population, (ii) the difference between statistical and biological significance, and (iii) the need to ensure that the methods and findings have biological relevance. As stated by Friedman (1980), "*no method of analysis, no matter how mathematically sophisticated, will substitute for careful evaluation of data based on good scientific judgment and knowledge of the disease process being studied*".

1.6 Summary

- Disease in wildlife is often of multifactorial causation, and the effects of many diseases on wild animals are understood poorly.
- Investigation of disease is undertaken for three basic reasons: to determine its cause, to determine its significance, or if justified, to identify methods for management.
- Disease may be studied in the individual animal or in the population; a combination of methods is usually required.
- Epizootiology involves the description and characterization of variables associated with disease in groups of animals and the comparison of factors among groups.
- Qualitative aspects of disease, such as the presence or absence of some factor, are evaluated by determining the proportion of the group affected, or the rate of occurrence. These rates form the basis for comparison among groups.
- Many features associated with disease occur on a continuous scale. These are described in terms of the distribution of occurrence and by measures such as mean and standard deviation. Association among factors is investigated by techniques for measuring correlation.
- Results of investigations must make biological as well as mathematical sense.

2 Special problems in working with free-living animals

"Usually, insufficient attention is paid to the infectious and parasitic diseases of wildlife until some outbreak of disease, no matter whether in wildlife or domestic animals, when the importance of disease or infestation of wildlife is often overestimated."

(Jansen 1964)

Although the same basic techniques are used for the study and management of disease in wild animals, domestic livestock, and humans, the wildlife specialist encounters difficulties that are unimportant or that can be controlled, literally or statistically, in studies of the other two groups. Most of these difficulties are a result of the 'wildness' of the subject animals. The word **wild** has many meanings, including "*growing without the care of man*", "*unaffected by civilization*", "*of great violence or intensity*", "*undisciplined*" and "*extravagant or fantastic*". No wild animal is unaffected by civilization, since all inhabitants of the globe share effects, such as chlorinated hydrocarbon residues and global warming, but most wild animals grow without (and some grow despite) the 'care' of humans. Most of the other definitions are applicable to free-ranging species. Disease is notoriously hard to detect, even in humans and domestic animals. Disease in wildlife has often been compared to an iceberg with only a tiny fraction or tip of the total mass being visible at any time. Part of this phenomenon is because very few people are looking for, and even fewer are reporting, disease when it does occur. However, the covert nature of disease, and particularly its quantitative aspects, makes disease inherently more elusive in wild animals than in either livestock or humans. The wildlife worker has a much greater difficulty finding diseased individuals than does either the physician or the veterinarian, and one is seldom able to count wild populations in the way that cattle in a pen or children in a school can be counted.

Arrival at an understanding of any disease is a slow, gradual process analogous to unwinding a ball of string comprised of many short lengths. Each bit of string removed, or fact discovered, brings one nearer to the core, so long as the fact is recorded and available to the next researcher. The study of disease in wild animals is a new science and there are relatively few scientists in the field, so that many of the facts taken for granted about humans and domestic animals remain to be discovered. The extravagant and fantastic

nature of wild species and their undisciplined response to various procedures create unique problems for those interested in disease, as does the relationship that exists between the public and wildlife.

2.1 Problems in detecting diseased animals

The most basic quantitative measure of disease in a group is an enumeration of affected individuals. In human and veterinary medicine, the detection of sick individuals depends upon the severity of clinical signs, the willingness or desire of the patient (or the owner) to seek and allow examination, the diagnostic personnel and facilities available, and the skill of the diagnostician. All of these factors also apply to the study of disease in wild animals but, in addition, disease detection is further complicated in this group by the difficulty of finding sick animals. In a few situations, disease may make affected wild animals overly available and this may cause problems of bias in samples. For example, rabies might make animals prone to be killed by automobiles, so that a sample of road-killed skunks may not be indicative of the actual prevalence of that disease in the population of an area. Similarly, Bellrose (1959) found that ducks that had ingested lead shot were more likely to be killed by hunters than were normal ducks. Hence, lead-exposed birds are likely to be over-represented within the hunters' bag. However, these examples are exceptional cases and, in most instances, sick animals become less rather than more readily available to the investigator. This is because of the restricted travel, secretive behavior, and increased susceptibility to predators that occur among sick animals. Predators and scavengers are usually in direct competition for specimens with the researcher, (but they may be cooperators in disease-management programs based on population reduction or carcass disposal).

When wild animals die, their carcasses are "*quickly assimilated into the environment*" (Stutzenbaker et al. 1986). The investigator who is measuring mortality based on counts of dead animals must consider two factors: (i) the proportion of the carcasses present that is detected, i.e., the efficiency of the search technique, and (ii) the rate of disappearance of carcasses as a result of decay and scavenging. Many descriptions of disease outbreaks contain estimates of the relationship between the number of animals found sick and/or dead, and the total number that died. For example, Hoff et al. (1973) suggested that the recovery rate of carcasses during an epizootic among deer in North Dakota was "*not more than 10%*" and then multiplied the number of deer found dead by a correction factor of ten to obtain an estimate of total mortality. However, these authors did not provide information on how the estimate of a 10% recovery rate was determined or of its accuracy. Other investigators have attempted a more quantitative approach to deal with this problem. Following a similar epizootic among deer in Montana, Swenson (1979) searched a large area and found 34 carcasses. He then marked the 14 carcasses found on a portion of the area and asked hunters to record and mark all

carcasses that they encountered on this portion. (This technique is a form of the classical mark-recapture method used widely by biologists for estimating animal numbers that will be discussed in Chap. 5). Hunters found 51 carcasses, including the 14 marked ones, at the area. Swenson used the ratio 14/51 to calculate that his search had located (at *maximum*) 27% of the carcasses present on the area. He adjusted the count on the overall area by a correction factor of 3.6 (51/14) to estimate that a *minimum* of 124 deer died at the entire area.

Some researchers have examined the efficiency of carcass searches experimentally (Robinette et al. 1954; Anderson 1978; Humberg et al. 1986; Stutzenbaker et al. 1986; Ward et al. 2006). Stutzenbaker et al. (1986) studied the effectiveness of search crews in locating dead ducks in a shallow Texas wetland. One hundred banded duck carcasses were distributed in a 40-ha marsh, with 50 of the birds placed in "*typical escape cover*" to simulate birds dying of chronic lead poisoning and 50 placed in "*completely exposed positions atop vegetation*" to simulate ducks that died of rapidly fatal avian cholera. Within 30 min of placing the carcasses, eight searchers (unaware of the carcass placement) were asked to search the area and to collect sick and dead birds. The searchers failed to find any of the birds placed in cover and found only six (12%) of the "*highly visible*" carcasses. The authors concluded that "*lack of carcasses recovered during intensive searches does not rule out extensive waterfowl mortality. Thus, casual searches would result almost invariably in negative findings even though large numbers of birds actually died.*" We found that the success in finding carcasses of ducks that had died of botulism during carcass searches is highly variable and as few as 7% of the marked carcasses may be recovered on large wetlands.

The rate of disappearance of carcasses also has been studied under a variety of circumstances (Wobeser et al. 1979b; Humberg et al. 1986; Stutzenbaker et al. 1986; Pain 1991; Wobeser and Wobeser 1992; Cook et al. 2004; Ward et al. 2006). Although the results of these studies were somewhat variable, it appears that about 50% or more of duck to goose-sized carcasses disappear within 4 days, and that 75% of passerine bird carcasses may be removed within the first day. Given this information, it is not surprising that smaller animals such as passerine birds, rodents, and neonatal ungulates, are seldom found dead. An exception to this rapid rate of carcass disappearance may occur when a large number of individuals die within a short period of time in a small area. The resulting plethora of carcasses appears to temporarily overload the normal removal mechanisms (Linz et al. 1991) and individual cases may persist for an extended period. We have found this to be true of duck carcasses marked and observed during a botulism outbreak. In one such situation, only 1 of 42 duck carcasses was disturbed by scavengers during the first 4 days after death (Cliplef and Wobeser 1993). If carcasses are removed rapidly by scavengers, it is obvious that mortality surveys based on regular, e.g., weekly, counts of dead animals contain a very significant underestimation bias.

Any detailed study of diseases that is based on the recovery of sick or dead animals should address these problems and attempt to measure the efficiency of the data collection methods used. Raw counts without some adjustment

cannot be used to calculate **absolute** morbidity or mortality, but could be used to monitor **relative** changes, as between years, providing that other factors remain constant.

2.2 Problems in determining numbers and identifying individuals

Epizootiology is a quantitative and comparative science. In human populations, at least in developed countries, and to a lesser extent in domestic animals, population parameters are obtained by **census.** This implies an actual count of all individuals, together with collection of details such as the sex and age composition of the population. It is seldom possible to census wild animals completely, except under unusual circumstances. Such circumstances may occur when a small number of conspicuous animals are located in an isolated area, e.g., the wolves and moose of Isle Royale, Michigan (Mech 1966), or when a highly visible species congregates in a small area, e.g., the mid-continent population of sandhill cranes on the Platte River in Nebraska (Buller 1979). In other situations, the person interested in wild animals usually must make do with an **estimate** of the number of animals in the population. An estimate may mean either a guess (an opinion without sufficient evidence) or a term referring to an average and its range of values as determined by a set of rules (statistics) (Davis and Winstead 1980). Unfortunately, many of the estimates used in wildlife disease work have been of the guess-type and wildlife biologists often must deal with population estimates that are of unknown reliability or that have very wide confidence limits.

Statistical estimates are obtained by sampling. The techniques include methods such as counting animals on a portion of the total area with quadrat or transect surveys, measuring some type of ratio of abundance such as the number of pheasants heard crowing/hour or the number of birds seen/km of road, or by using an index to abundance in which some object associated with the animal is counted rather than counting the animals, e.g., counting muskrat houses rather than muskrats. Methods for measuring population parameters will be discussed in detail in Chap. 4. At this point it is important to recognize the difference between a census and an estimate as well as to realize that even ‘good’ estimates of population size in wild animals often have wide confidence limits. The latter factor becomes problematic when trying to assess the impact of disease on a population or to assess the effectiveness of some management. For instance, if the best possible estimate of population size has confidence limits ±30% of the mean, it will be difficult (or impossible) to detect the impact of even major disease events on the population. Harding et al. (2005) provide an example of the extent of sampling that is needed to detect changes as great as a 60% decline in the population of some species.

Humans have names, social security numbers, and other features that identify us as individuals. Domestic animals are usually identifiable by tags, brands, tattoos, or by linking them to their owner. In contrast, wild animals are anonymous, except for a tiny proportion of the population that may have been marked by biologists. This means that one powerful tool commonly used for the study of disease in humans and domestic animals is impractical for the study of many free-ranging species. In human and veterinary medicine, individuals often can be traced backward in time to determine if they have been exposed to a certain factor, or forward in time to determine the outcome of exposure to a factor. Thus, if we are interested in the relationship between smoking and heart disease, we might determine, through questioning, the smoking history of a group of cardiac patients. Alternatively, we might follow a group of smokers for several years to compare the incidence of cardiac disease in this group relative to that in a similar group of non-smokers. Such retrospective trace-backs and prospective studies are seldom possible in wildlife. For example, a mallard found dead in a pond in Saskatchewan in early spring might have just arrived from a wintering area located anywhere between Florida and California. There are no distinguishing features or marks on the bird to indicate its recent past and there is no simple way to trace back to see if it may have been exposed to a pesticide in Arkansas, an avian cholera outbreak in Nebraska, or duck plague virus in California. In such cases, all one can do is perform specific analyses to look for residues or antibodies to each of the possibilities. Looking for residues or antibodies is like looking at animal tracks in the mud, the tracks tell you that something has passed but only an expert tracker can estimate when the event occurred. The results of analyses may indicate past exposure but will not tell when or where the exposure occurred. Similarly, it would not be possible, without a massive marking and monitoring program, to follow the fortunes of a group of birds that were exposed to a particular disease agent at a specific site. In wild species, one seldom is able to follow the clinical progression of naturally occurring disease in an individual, and most diseased individuals are not detected to be sick until they are in extremis or dead. A method that can be used to follow animals forward in time is through the use of radiotelemetry. For instance, Moriarty et al. (2000) followed 247 radio-marked adult rabbits for a year in Australia and found that the overall mortality rate of 82% was comprised primarily of deaths caused by predation (44%), rabbit hemorrhagic disease (16%), and myxomatosis (9%).

In general, humans and domestic animals are rather sedentary, while many wild animals are highly mobile. When dealing with sedentary species, the investigator can be reasonably confident that he is looking at approximately the same population from week to week. This is not the case with mobile (and especially migratory) wildlife. During a study of avian cholera in geese in Saskatchewan, we measured the size and species composition of the goose population on a study area by conducting weekly aerial surveys (Wobeser et al. 1979b). We were able to determine the approximate age

distribution within some species by counting the number of juvenile and adult birds within flocks. Thus, we were able to estimate the overall population and its approximate composition each week; however, we could not tell which individual birds were present from one week to the next. It is obviously very important to collect this type of information if the length of exposure to some factor is important in a disease. In this example, and in many other situations involving mobile wildlife, periodic estimates of population are analogous to a series of still photographs, taken from above, of a revolving door in a busy building. The number of individuals within the doors in each photograph can be counted, but it is unclear whether the faceless individuals are going round and around, i.e., a sedentary population, or if new persons are continually passing through in one or both directions. Lehnen and Krementz (2005) used a sample of radio-marked birds to estimate the average time that pectoral sandpipers spent on a staging area and the degree of turnover in the population with time, and used this information to assist in estimating the total number of sandpipers passing through the site during migration.

The ability to distinguish between residents and transients is usually critical in disease investigation. This is particularly true when trying to evaluate the effects of short-lived phenomena, such as a pesticide application. For example, one method for evaluating the effect of a pesticide spray program on birds might be to count birds in the area immediately before and then again a few days after spraying. If the population size remained approximately constant before and after spraying, this might be interpreted to mean that the spray had no effect. However, the same findings would occur if some, or all, of the population present at the time of spraying died but were replaced by new birds not exposed to the toxin. In such a circumstance, it would be critical to be able to differentiate between residents and transients. It might be necessary to capture and mark a large number of the birds on the area prior to the spray application, and then do a recapture program to confirm that these individuals were still present after spraying, in order to identify population turnover.

Another difficulty that may be encountered when working with mobile wild species is that disease may occur only during a portion of the year when the animals are inaccessible or difficult to observe. For instance, heavy infestations with the flea *Ceratophyllus vagabundus* occur on lesser snow and Ross' geese while they are nesting in the arctic (V. Harriman, personal communication) but I have never observed a flea on any of the many Ross' and snow geese that I have examined during spring and autumn migration. Similarly, it is difficult to evaluate the effects of oil pollution on seabirds, because the birds can only be counted on breeding areas, while oil spills usually occur in remote wintering areas among birds dispersed over vast areas (Votier et al. 2005). Population dynamics of a migratory species may be influenced by factors encountered at a staging area that is visited for only a short period of time (Schaub et al. 2005); if the factor is a disease agent and it is not evaluated at that site and time, its effect will not be detected.

2.3 Problems related to lack of knowledge about the animals

Accumulation of knowledge is a gradual process and the study of disease in wild animals is only of recent origin, so it is not surprising that many pertinent facts are unknown. The quantitative study of disease in human populations dates back almost four centuries to the Bills of Mortality collected in London and Hampshire beginning in 1603 and analyzed by Graunt in 1662 (Lilienfeld 1980). In contrast, no comparable catalogue of mortality exists for any wild species, except perhaps for a few endangered species within recent years. The statement that "*the most elusive vital rate in population dynamics is usually natural mortality*" (Lett et al. 1981) is particularly appropriate for wild animals. Such studies usually require the use of special techniques such as radiotelemetry, as in the study by Moriarty et al. (2000). It is encouraging that information on the occurrence of certain diseases in wild species is now being collected systematically by the National Wildlife Health Center, U.S. Geological Survey in the USA, and the Canadian Cooperative Wildlife Health Centre in Canada. The importance of recording and maintaining such data will be discussed in detail in Chap. 7.

The lack of information about wild species applies not only to the quantitative aspects of population but also to basic questions of natural history, behavior, anatomy, and physiology. The basic nutritional requirements of the domestic cow, horse, and chicken are well defined and readily available, but a researcher interested in nutritional disease in shrews, chipping sparrows, or bullfrogs has no handy reference source. An investigator may obtain some information of this type by cooperating with experienced biologists but often basic information, such as food habits, home range, gross and microscopic appearance of normal tissues, concentration of blood constituents, expected parasite fauna, and age at first reproduction, any of which might be critical for understanding a disease, will be unavailable and will have to be collected by the investigator. It is well to recognize in advance that the study of disease in a wild species often must include a substantial investigation of the basic biology of the host species to fill in the missing baseline information.

In the absence of specific information, data and techniques derived from humans and domestic animals often are applied to wildlife. This is a logical first step and may be suitable when the extrapolation is over a short distance, such as from domestic to wild turkeys. However, the investigator must always be aware that extrapolation is risky and may not be appropriate, e.g., domestic Pekin ducks, game-farm mallards, and wild mallards react differently to certain pathogens, although all are *Anas platyrhynchos*. Verification of the suitability of the data or technique must be a high priority. Serologic tests developed for domestic animals are often applied to wild species without any knowledge of the specificity or sensitivity of the test in the wild animal. When such methods are tested, they are often found to be inappropriate. For instance, Thorne et al. (1978) found that none of the serologic tests used for diagnosing brucellosis in cattle was adequate by itself for diagnosing the

disease in elk. Nielsen et al. (2005) provide a good description of the shortcomings of using unvalidated tests in wild animals. Application of techniques from domestic species to wild animals may not only be inappropriate but also may be dangerous, e.g., a modified live virus vaccine that is used routinely for the immunization of dogs against canine distemper was fatal disease when used in endangered black-footed ferrets (Carpenter et al. 1976).

2.4 Problems related to the diversity and intractable nature of wild animals

Physicians deal with one species, veterinary practitioners deal with less than ten species, whereas the wildlife disease specialist must be knowledgeable about a multitude of species that occupy diverse ecologic niches. A single incident, such as a spill of a contaminant into a marsh, may affect 100 vertebrate species, including fish, amphibians, reptiles, birds, and mammals. None of these animals will have exactly the same susceptibility to the compound and each may have a different clinical expression of intoxication. It is probable that neither the susceptibility nor the clinical response will be known for any of the species involved. Because of the complexity of the situation, the investigator may be forced to choose a few species to serve as indicators for the entire range of animals. Inappropriate indicator species may lead to either over- or underestimation of the impact of the contaminant. The risks in extrapolating from domestic species have been discussed, but it must be remembered that even similar, closely related wild species sharing an environment may react totally differently to a disease agent. For instance, red grouse, ptarmigan and willow grouse develop fatal disease when exposed to the louping-ill virus, whereas black grouse, capercaillie and ring-necked pheasants found in the same area have a mild, subclinical response to this agent (Reid et al. 1983). Similarly, the northern pintail is highly resistant to duck plague, while the blue-winged teal is exquisitely susceptible (Spieker 1978, Wobeser 1987), although both are dabbling ducks of the genus *Anas*. The high degree of variability in the response of various birds to DDT (Blus et al. 1972) resulted in confusion and controversy as to whether thinning of eggshells was a real phenomenon, and if there was a cause-and-effect relationship between the thin eggshells and the pesticide. This resulted in unnecessary delays in control of the DDT problem.

The problems encountered in finding and counting wild animals have been discussed earlier. The manipulations applied to these animals after they have been found also may produce a range of difficulties not experienced by those working with humans or livestock. These effects may confound the interpretation of results of a study and endanger the health or

life of the species being investigated. In some species, the mere presence of an investigator or observer may in itself be a major morbidity or mortality factor. This effect is best documented in colonial birds, but harassment of the type required for a study may have an undesirable effect on many bird species. King et al. (1977) described nest abandonment by brown pelicans because of heavy infestations with *Ornithodoros* sp. ticks and suggested that this disease could be an important factor influencing the nesting success of the bird. However, these authors concluded that they could not collect more information about this disease "*without seriously disturbing and destroying large numbers of nests*" of this threatened species. Other researchers have been less sensitive and perceptive. Many reports contain statistics such as nest success, hatching success, and the rate of chick mortality, without accounting for the impact of the researchers' interference on the birds.

The assumption is made in many studies that samples collected from captured wild animals are representative of those in the population and that capture, handling, marking, and other manipulations have no effect on the samples or on subsequent life or survival of animals after release. In most instances these assumptions are untested. Each person who captures, restrains, or handles a wild animal for study should be aware that the manipulation may induce a spectrum of perturbations in the animals. These may range from minor changes in blood constituents to fatal capture myopathy (degeneration of muscle). Kock et al. (1987a, 1987b, 1987c) presented a detailed description of the effects of capture in one species. It is probable that most wild species react in a similar manner. Over-reaction to even simple procedures is a dark side of working with wildlife. The effects may confound data and result in spurious conclusions such as biasing estimates of mortality rate (Abbot et al. 2005), and endanger the subjects. Reduction of such effects should be a part of the planning of every project. For example, treatment with vitamin E and selenium at the time of capture improved the long-term survival of the northern bobwhite (Abbot et al. 2005).

Many investigations of wildlife disease include some type of laboratory study and, because laboratory-bred animals are not available in most instances, animals captured from the wild are often used as subjects for such studies. One need only compare such wild-caught animals with the highly inbred, standardized strains of the usual laboratory animals to realize the variability and difficulty in having appropriate controls for such experiments. Wild animals brought into the laboratory may develop a variety of spontaneous and unexpected conditions (as a response to captivity) that may interfere with the experiment. For example, herring gulls appeared to adapt to captivity behaviorally but developed anemia and amyloidosis that interfered with their suitability as experimental animals (Hoffman and Leighton 1985).

2.5 Fitness, trade-offs, and predators

When considering disease in humans and most domestic animals, we are usually concerned with the effects of one agent or factor at a time, but in wild animals, multiple potential disease agents are often present and interact, making separation of the effects very difficult. The ecological concept of fitness, i.e., relative lifetime reproductive success, is largely irrelevant in humans and domestic animals. Fortunately, few humans in an overcrowded world are concerned with maximal reproductive output, and most domestic animals live an abbreviated life or are neutered. However, in wild animals, we must consider the effects of disease on lifetime reproductive performance. Fitness has two components, survival and fecundity, each of which may be influenced by disease. Even subtle effects may have important population consequences. For instance, the cowpox virus produced no visible disease in rodents and did not affect their survival, but infection with this virus delayed the onset of reproduction by a month, resulting in a reduction of about 25% in the reproductive output of these short-lived animals (Feore et al. 1997).

In humans and domestic animals, we are usually concerned with the direct effect of disease agents on survival or quality of life and we don't worry that disease will make us more vulnerable to tigers, or that disease might make our cows more vulnerable to wolves or our chickens more vulnerable to hawks. In contrast, even mild dysfunction, as a result of subclinical or sublethal disease, may indirectly affect survival of wild animals through increased vulnerability to other factors, notably predators (Temple 1987; Kavaliers and Colwell 1995; Ives and Murray 1997). The concept of physiological trade-offs among resource-demanding functions (Stearns 1992) provides a framework for considering interactions among disease agents and other factors such as predation, nutrition, and inclement weather. Wild animals must allocate limited resources among maintenance, production (including growth and reproduction), and storage. If an animal must use scarce resources to defend against disease or to repair damage caused by disease agents, less resources will be available for reproduction, predator avoidance, or maintenance under inclement conditions. The affected individuals then may be at a selective disadvantage within the population. The interactions can be unexpected, and even the cost of mounting a successful defense against a potential disease agent can have serious consequences. For instance, mounting an immune response to an innocuous antigen may lower reproductive output (Ilmonen et al. 2000) or reduce long-term survival (Hanssen et al. 2004). Conversely, exposure to predators may reduce the ability to mount an immune response (Navarro et al. 2004). Such indirect effects of disease may be important at the population level but be extremely difficult to detect.

2.6 Problems related to people

Wild animals in many parts of the world are a public resource and most people have some interest in their well-being. The relationship between the public and wildlife is complex, but some parts of the relationship are embodied in clichés such as 'wildlife belongs to everyone', 'no one owns wild animals' (to which a cynic might add 'particularly when there is a problem'), 'wildlife would be okay if we could just restore the balance of nature', or 'the wildlife situation was better when I was a child'. Many of the relationships are emotional and strongly held, so that the individual responsible for managing wildlife often faces a formidable task in gaining public approval for an action that may make eminent biologic sense.

The perception that all disease in wild animals is a natural phenomenon and that nature will take care of itself, is still prevalent. Disease, in general, is a natural condition of populations, but I cannot agree that specific conditions related to pesticides, acid rain, or mercury pollution, or that rabies introduced into an area with transplanted raccoons, are natural phenomena. Nor is it probable that such conditions will get better with no action. It is aesthetically painful to admit that human activities have a direct or indirect effect on every wild species but a realistic acknowledgement of this fact should be part of the consideration of any disease in wild animals. Because of the difficulties in finding diseased animals (discussed earlier in this chapter), and the subtleties of disease in wildlife, it is often difficult to convince the public (and politicians) that a problem exists that should be investigated or managed.

Lead poisoning of birds provides an example of this difficulty. That lead poisoning is an important mortality factor in wild waterfowl is incontrovertible, as is the fact that the disease eventually could be eliminated in many situations by the use of a non-toxic substitute for lead shot in shotgun cartridges. Despite this, introduction of non-toxic shot met with incredible resistance. Some of this resistance was from vested interests with an economic stake in retaining the status quo, but much of the resistance seemed to relate to resentment of interference in traditional hunting of waterfowl. Because of the chronic nature of lead poisoning in birds, most affected individuals crawl away to hide and to be consumed by predators, so the disease is largely invisible. This made it extremely difficult to convince hunters that a problem existed, because they did not encounter ducks dead of lead poisoning while slogging through the marsh. Few hunters seem willing to accept the word of a biologist if it conflicts with what they have seen with their own eyes. Compulsory use of non-toxic shot has been accompanied by tremendous ill-will and was accomplished in the USA only through use of the court system.

The chronic problem of inadequate funding of wildlife work, including that related to disease, does not need elaboration for an audience with first-hand experience. One of the unfortunate effects of this under-funding has been that most studies of disease are of short duration (usually the period required for a

graduate degree), whereas most diseases can only be understood after observation over an extended period. The collection and maintenance of records of disease occurrence in some form of data bank is a vital step toward gathering such information. Getz et al. (1987) provide an insightful discussion of the deficiencies of short-term studies of long-term phenomena. These authors studied populations of *Microtus* spp. over a 14-year period and failed to find convincing evidence of the population cycles long thought to be characteristic of this genus. By analyzing specific short segments of their data, they were able to produce patterns suggesting cyclic change, but concluded that "*most previous assumptions of multiannual cycles in these species may be artifacts of short-term studies.*"

We have come to expect that virtually all diseases of humans and animals can be prevented or cured through technology (vaccines, drugs, surgical transplants) and we forget that most of the actual advances in health have been accomplished by improved sanitation, clean water, and safe food. Lack of a simple technological solution may help to explain why AIDS has caused a panic. Medical science has also turned progressively toward high-tech research and population-based studies have fallen from favor. 'Magic-bullet' solutions will be infrequent for diseases in free-living animals. The major problem with chemical treatments and vaccines for wildlife lies in delivering them in the wild. Drugs and vaccines could work as well in wild animals as they do in humans or livestock; however, the problem lies in getting the animal and the therapy together. Solutions that do work will require very detailed knowledge of the population dynamics of both the animal and the disease agent. Unfortunately, studies of this nature currently enjoy relatively little favor with granting agencies. The need for an approach to disease in wild animals that is different than that used in humans and domestic animals may be difficult to explain to the public and even to some biologists. Some scientists have suggested that the inability to apply therapeutic drugs to wildlife means that nothing can be done about disease in these species. I hope to demonstrate that this is not the case. Effective disease management in wild animals will be primarily through manipulation of habitat or population factors for, as stated eloquently more than 70 years ago "*the real determinants of disease mortality are the environment and the population, both of which are being "doctored" daily for better or worse, by gun and axe, and by fire and plow*" (Leopold 1939). Acceptance of this type of approach to disease management requires a different perspective than that which has been customary in human and veterinary medicine.

2.7 Summary

- Disease is more difficult to detect and measure in wild animals than in human or domestic animal populations.
- Sick and dead wild animals are quickly removed by predators and scavengers and, hence, are unavailable to the investigator.

- Wild animals can seldom be counted. Quantitative information related to disease is usually dependent on estimates rather than counts.
- Wild animals are anonymous, so it is seldom possible to determine if individual animals have been exposed to causative factors in the past, or to monitor disease occurrence in individuals after exposure to a risk factor.
- Information on basic factors that influence disease such as food habits, nutritional requirements, behavior, normal biochemical values, and susceptibility to various agents often is unknown in wild animals.
- Techniques needed for disease investigation such as observation, capture, handling, and specimen collection, often result in dramatic physiologic and behavioral changes that may confound the results of the investigation and even endanger the life of the wild animals under study.
- The effects of disease in wild animals are often indirect but may have serious consequences for the animal's lifetime fitness. There are important interactions between disease and other factors such as predation, which result from the need to allocate physiological resources among competing needs.
- Public attitudes toward wildlife and toward disease in these species often interfere with investigation and management.
- Treatment and immunization, which are standard methods in human and livestock medicine, have limited application in wildlife because of difficulties in delivering the therapy to the animals.
- Management of disease in wild animals will take place primarily through manipulation of habitat and population factors.

Section II
Disease investigation

"*To do science is to search for repeated patterns, not simply to accumulate facts . . .*"

(MacArthur 1972)

3 Identifying and defining a disease

> "*An epidemiologist is one who thinks in an epidemiological manner, rather than one trained in one specific discipline. He must be a master of 'lateral thinking', trying to see connections between what are probably isolated observations of completely different natural phenomena*"
>
> (Halpin 1975)

There are many reasons for investigating disease in wildlife but in the simplest terms these usually resolve to some combination of: is disease present? what is causing it? and what effect is it having? In some cases, there may be a marked urgency to the study, as when it is necessary to determine the cause of a major outbreak of disease so that control measures can be instituted. In other instances one can take a slightly more leisurely approach, as when there is a need to assess if some disease factor is associated with an observed change in a population or to assess the significance of some potential risk factor such as an agricultural pesticide. Regardless of the reason for study, an early step in any investigation must be to define the problem to be investigated as precisely as possible. The objectives in defining the disease are to identify those features that distinguish the particular condition from all others, to delimit current knowledge about the disease, to identify questions about the disease that need to be answered, and to determine those methods that will be most likely to yield pertinent answers. The definition of a disease must be looked upon as a touchstone to which all aspects of the investigation are referred. As new information is discovered, the investigator must continually ask if it is part of the condition under study. Defining a disease is a dynamic process, and the definition is refined as new information is obtained. In the early stages of an investigation, the definition often will be very general but it should become increasingly precise as the investigation proceeds.

Although it may seem simplistic, a disease is defined by answering the same questions that cub reporters are told to ask when researching a story: **who? where? when? what?** and **why?** In more technical terms, these translate to: the population characteristics of affected animals (who?), location and environmental factors associated with the disease (where?), temporal distribution of the disease (when?), clinical, pathological and other analytical features of both the disease and the putative causative factor (what?), and the pathogenesis (why?). Pathogenesis includes both why the disease occurred in the individual,

as well as why or how the disease occurs in the population. Population and environmental factors will be discussed in detail in Chaps. 4 and 5, respectively. The remainder of this chapter will deal with defining the time, place and cause.

3.1 Temporal distribution of disease

Time is a quantitative scale against which all other aspects of disease can and should be measured. Rates that are used commonly to describe features of disease, such as incidence and mortality, have a time component and plotting of events on a time scale should be a basic part of every disease study. In constructing such a 'time graph', the investigator is looking for specific patterns or features, such as clustering of events in time, associations between different events in time, diurnal, seasonal or cyclic periodicity of occurrences, and changes in the disease over time. The appropriate time scale, against which other factors should be measured, may vary from seconds to decades, or even to millennia. An example of the latter time frame would be a situation where one is attempting to understand the evolutionary aspects of a host/parasite relationship or using molecular genetics to unravel the past distribution of an organism.

Clustering or dispersion of events, such as mortality or morbidity over time, often provides valuable clues to the nature of a disease. The classical use of this type of temporal data is in the construction of an **epizootiologic curve**, in which the number of new cases occurring in a population, or of animals dying in an area, is plotted against a time scale. The resulting graph or curve plotted in this way illustrates the incidence rate of the disease, as well as changes in the pattern of occurrence (Fig. 3.1). Certain patterns of disease

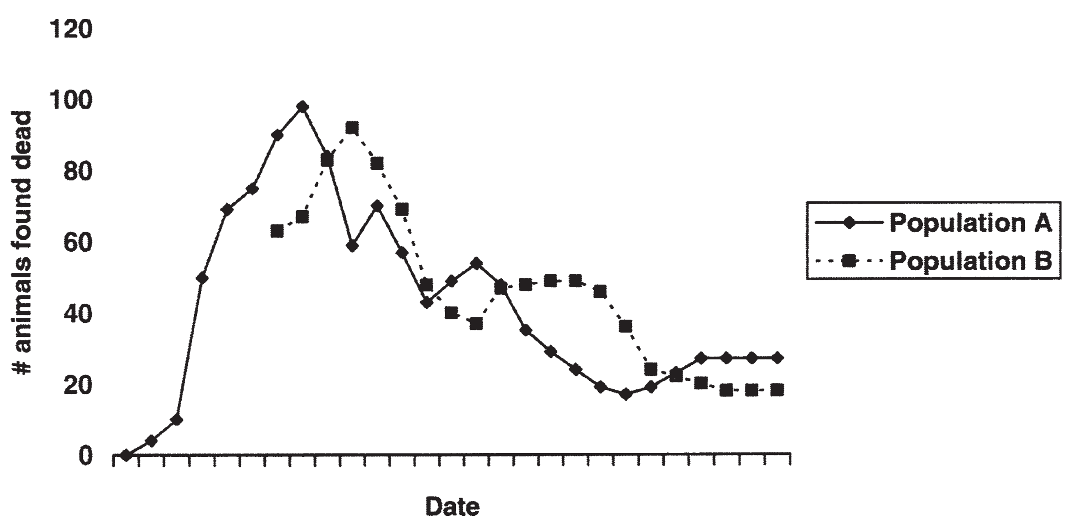

Fig. 3.1 Epizootic curve for a disease outbreak that involved populations of the same species in two locations. As often occurs in wild animals, the disease was likely present in population B for an unknown length of time before it was recognized

occurrence have become so well recognized that they are considered indicative of a particular method of disease transmission. For example, Adrian et al. (1978) described outbreaks of a fungal disease (aspergillosis) in ducks, in which the mortality began suddenly and lasted for less than 7 days. A graph of the number of cases per day of such an occurrence would have a single sharp peak. This pattern is considered typical of a **point** or **common source** outbreak, and suggests that all of the affected birds were exposed to a single source of infection over a short time period. In the aspergillosis outbreaks, the point source in 1 year was mouldy ensilage that had been used as feed by the ducks for a few days because of deep snow in the area. If this source had been available and used over a longer period of time, one would expect a more prolonged and less well defined peak of mortality. In contrast, Roughton (1975) described an epizootic among deer in Kentucky in which there was a bimodal pattern of mortality over time. This pattern is compatible with that produced by an infectious agent spreading among the population. The time period between the first peak (primary cases) and the second peak (secondary cases) may give an indication of the average incubation period of the disease.

The appropriate time scale for constructing an epizootiologic curve (or any other temporal measurement) may be based either on calendar time or on absolute time from some event. For instance, if we were interested in the effects of pesticide applications on birds in an orchard, we might plot the number of affected birds observed and the date of spray applications on a calendar-based scale. The resulting pattern (Fig. 3.2a) suggests that there is a strong association between spraying and the observation of sick birds. Each of the peaks is of the common source type, and this pattern might be classed as a common source, multiple event type. If the same information is plotted on an absolute scale, based on time after spray application, the results would appear as a single peak, assuming only a single pesticide was involved (Fig. 3.2b). If two different chemicals had been used, each of which caused morbidity but after different time intervals, the results might appear as a bimodal pattern when plotted in this way.

If one was interested in the occurrence of disease among nestling colonial birds, it might be wise to plot mortality against both calendar date and on an absolute scale based on days after hatching, as the two graphs might reveal different associations. The calendar based scale should reveal association with events such as inclement weather or harassment of the birds (Fig. 3.3a), while the other scale may demonstrate associations between disease occurrence and age (Fig. 3.3b).

In some instances, the association between an event and the occurrence of disease is obvious, as when a die-off occurs within hours or days after the application of an acutely toxic pesticide in the area (e.g., Bailey et al. 1972), or when two or more events occur simultaneously, such as when geese on a hypersaline lake became encrusted with salt coincident with a sudden fall in temperature (Wobeser and Howard 1987). In other situations there may be a long delay between exposure to the causative factor(s) and the expression of

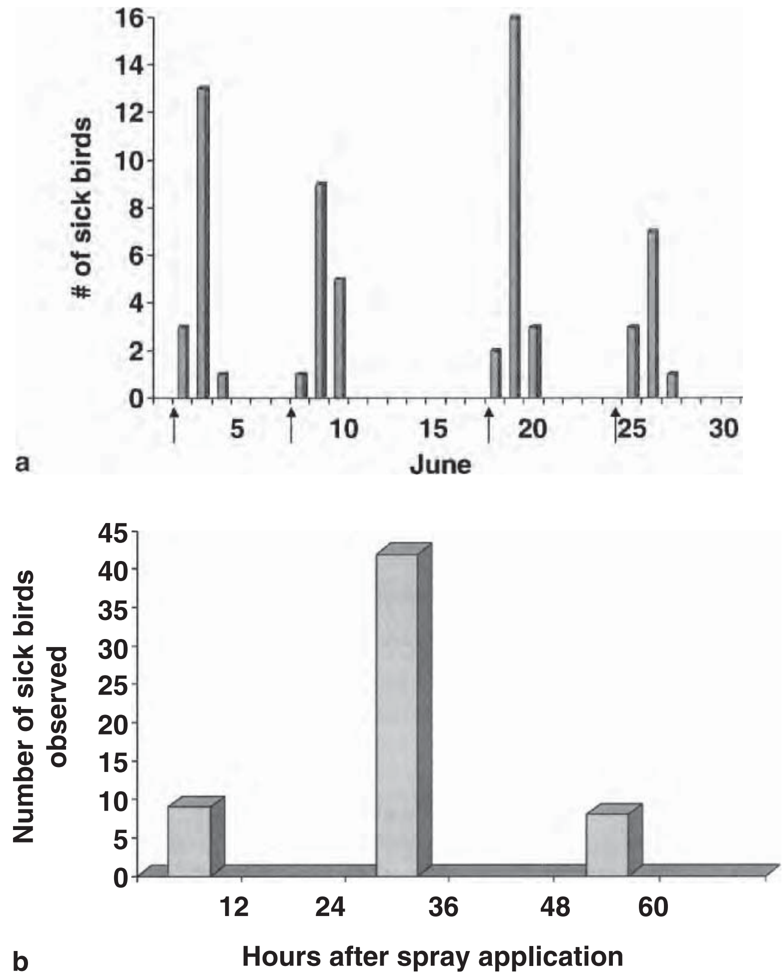

Fig. 3.2 Temporal relationship between morbidity of songbirds and applications of pesticide in an area (hypothetical data). In **a**, the time scale is based on calendar date and an association is evident between spray applications (arrows) and the observation of sick birds. In **b**, the data have been pooled and expressed in terms of absolute time after spray application

disease, so that the temporal association between the two events is not obvious. For example, the effects of chlorinated hydrocarbon insecticides on reproduction by golden eagles in Scotland occurred long after the birds were first exposed to the chemicals. However, even in such instances, temporal comparisons may be useful. Lockie (1967) and Everett (1971) compared the level of usage of chlorinated hydrocarbon chemicals, concentration of insecticide residues found in eggs, and reproductive success of eagles over a period of years and, in doing so, demonstrated a clear association among these factors over time. There often is a delay between different events in infectious diseases. For instance, the peak prevalence of antibody to hantavirus in rodents populations occurs after the population has peaked and has begun to decline (Calisher et al. 2005). A 2-year lag between certain weather conditions and increases in human plague has been observed (Enscore et al. 2002) and the strongest predictors of risk of Lyme disease for humans were the abundance of small rodents in the prior year and the abundance of acorns 2 years previously (Ostfeld et al. 2006).

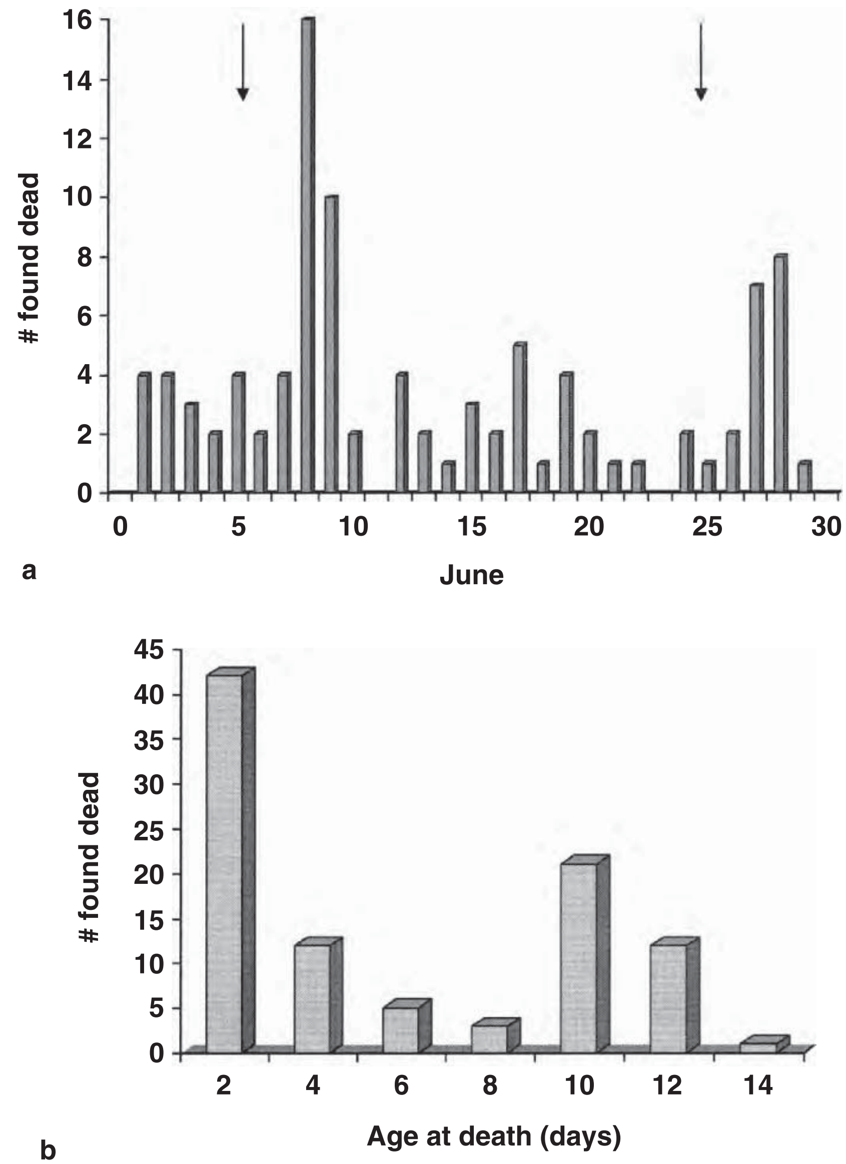

Fig. 3.3 Two methods of measuring the temporal aspects of mortality of young birds of a colonial nesting species. In **a**, the number of birds found dead is related to the calendar date and an association between storms (arrows) and mortality is evident. When the same data are expressed on an absolute time scale, based on age of the birds at the time of death (**b**), there appears to have been a bimodal pattern of mortality with one peak occurring immediately after hatching and a second at about 10 days of age. This suggests that two different mortality factors were acting in the colony

A large number of factors may be related temporally and the relationship among all the factors may only become evident when they are superimposed on a time scale. Disease in ducks caused by the protozoan blood parasite *Leucocytozoon simondi* is an excellent example of this. The intricate choreography of the host:parasite relationship in this disease only became evident when the timing of weather changes, emergence of various blackfly species that act as vectors, migration and reproduction of ducks, duckling development, and the life cycle of the parasite in both the fly and duck host were considered simultaneously (Fallis and Bennett 1966).

Many diseases have a distinct periodicity and these changes may be diurnal, seasonal (periodicity of 1 year), or cyclical (periodicity of several years). For example, blood parasites often have diurnal variations in their frequency of occurrence in the peripheral circulation, presumably so that they are readily available to suitable arthropod vectors that also have diurnal periods of activity. Seasonal variations, which occur annually, are probably the most commonly observed type of disease periodicity in wild animals. For example, we have come to expect specimens of certain diseases to be submitted to our diagnostic laboratory at definite times of the year. In the spring, we expect to receive cases of avian cholera in geese and eagles poisoned as a result of the illegal use of pesticides to poison coyotes (Wobeser et al. 2004). During summer, cases of botulism in waterfowl, poisoning of birds associated with spraying for grasshoppers, and corvids suspected to have West Nile virus infection are submitted. In autumn, we have come to expect submissions of pronghorns with polioencephalomalacia, hunter-killed moose and ducks submitted because of parasites, and emaciated juvenile raptors and herons. During winter, predictable submissions include starved deer, tick-infested moose, and coyotes, foxes and wolves with sarcoptic mange. The timing of submission of specimens to our laboratory may be because of a genuine seasonal occurrence of the disease or for other reasons. In some of the above cases, it is easy to explain seasonality of the submissions, e.g., acute pesticide poisoning occurs when the compounds are used, winter ticks (*Dermacentor albipictus*) are only present on moose during the colder parts of the year, and winter survival is a limiting factor for many northern ungulates. Others among this group also appear to be genuine seasonal occurrences, but the reasons for these are not so clearly defined. Polioencephalomalacia, a degenerative disease of the brain, might be related to consumption of grain (Wobeser et al. 1983; Wobeser 1984), which is more common among pronghorns in the autumn than at other times of year, or it might be related to consumption of water high in sulfates from wetlands in which salts become increasingly concentrated during summer and autumn. Botulism may be most common in late summer partially because the temperatures at that time of year are most suitable for growth and toxin production by the bacterium but also may be related to a wide variety of other variables including the concentration of moulting birds on botulism-prone wetlands at this time of year. The occurrence of emaciated hawks, owls, and herons in the autumn is probably because these are young-of-the-year birds that are unable to feed themselves after leaving parental care. Other submissions to our laboratory are examples of spurious periodicity that result from an increased probability of detection at one time of year. For example, parasites in moose meat and cysts of *Sarcocystis* sp. in ducks are submitted in large numbers in autumn because this is the season when hunters are active in the field and handling game meat.

The cyclic occurrence of population changes in wildlife, i.e., those recurring with a periodicity of several years, including those related to disease, is a controversial subject and beyond the scope of this book. The reader interested in

this subject should consult authors such as Anderson (1981, 1982) and Anderson and May (1982) for a discussion of a theoretical basis for cyclic behavior in relation to disease, and Hudson and Dobson (1995) and Hudson et al. (1998) for details of the relationship between parasitism and cyclic population behavior. From a practical point of view, detection of such cyclic behavior requires the consistent application of standardized methods of data collection and measurement to a population over an extended period of time; something that has been done very rarely in the study of disease in wild populations. A study by Getz et al. (1987) of *Microtus* spp. illustrates the risk inherent in trying to understand cyclic population changes through short-term studies.

Diseases may also change in a non-cyclical manner over time. Such changes are referred to as secular changes or trends. Major changes, such as the decrease in pathogenicity that has occurred in myxomatosis in rabbits in Australia (Kerr and Best 1998) or the changes that have occurred in some bird populations following introduction or suspension of use of certain pesticides, may be relatively easy to detect. However, such radical changes are undoubtedly the exception and other more subtle but perhaps equally important changes go undetected without careful research and diligent record-keeping. A long-term study in England of the population effects of agricultural modernization and chemical use on the grey partridge (Potts 1978) provides an example of the benefits of such research. Reed and Plante (1997) found a significant decline in body size, mass, and condition of greater snow geese over a 20-year period, resulting from changing conditions on arctic brood-rearing areas, and predicted an increase in juvenile mortality during migration. A study of amphibians by Pechmann et al. (1991) illustrates the need for studies that span many years to differentiate between natural population fluctuations and a long-term population decline. In that study, salamander and frog populations were monitored daily, using a standard method, over a 12-year period. During this period, there were substantial fluctuations in population size but no evidence was found of the overall population decline that had been reported from many short-term studies.

3.2 Spatial distribution of disease

Variations in disease occurrence and frequency according to place have been recognized since antiquity. **Definition of the spatial limits and the distribution within those limits of factors related to the diseases should be part of every disease investigation.** The scale of measurement may vary from geographic distribution by country or continent to very minute foci defined by subtle changes in microclimate. Regardless of the scale and degree of sophistication, the basic procedure in all instances is some system for plotting events and factors related to the disease on one or more maps. The actual

procedure may vary from simply marking the location of dead animals on a sketch map to describing events in terms of mathematical coordinates that can be analyzed using geographic information systems (GIS). The important feature is to arrange all of the information that is available in a manner so that spatial relationships among factors are apparent.

Spatial distribution should be considered in a three-dimensional sense, i.e., latitude, longitude, and altitude, as the distribution of a disease may be restricted in any one of these planes. For example, ducks held in cages 2–5 m above ground level rarely became infected with the parasite *Leucocytozoon simondi*, whereas infection was consistent and severe in ducks placed at ground level (Fallis and Bennett 1966). This distribution of the disease was a result of the foraging habits of the blackfly vector.

The spatial distribution of disease factors must always be considered in conjunction with time, and changes in distribution over time are also important. Statistical methods for measuring clustering of events in time and space are available (Williams 1984). The use of some of these methods in the investigation of animal disease is well illustrated in a study by White et al. (1989). The spread of rabies through western Canada (Tabel et al. 1974), Europe (Bogel et al. 1976), and parts of Latin America (Lord 1980), and of rabbit hemorrhagic disease in Australia (Kovaliski 1998) have been documented by mapping distribution against time. The ability to predict the direction and the rate of movement of the disease has been critical in the development of control programs for rabies. The abrupt halt of an advancing front has been used to measure the effectiveness of skunk control (Rossatte et al. 1986b), vaccination of foxes (Steck et al. 1982) and vampire bat control (Lord 1980) in controlling epizootics.

The geographic range of a disease is the most basic of spatial information. Even a large-scale map showing the approximate location of all known cases may provide useful information. For example, a wildlife manager in a western province or state could use a map such as in Fig. 3.4 to determine areas from which it would be unwise to allow importation of white-tailed deer, because of the potential of importing the meningeal worm *Parelaphostrongylus tenuis* not present currently in western areas. Similarly, a biologist contemplating translocation of moose or caribou into the known infected areas would benefit from this type of information, as such transplants into enzootic areas have had poor success.

Although this type of information seems elementary, mistakes based on lack of knowledge of the geographic range of disease are common. For example, woodland caribou from Newfoundland were translocated to Maine in 1986. This was a mistake for two reasons: the first was that caribou in parts of Newfoundland are infected with the nematode *Elaphostrongylus rangiferi*, which is not known to be present in any other location in North America. It affects not only caribou/reindeer but can cause severe disease in moose (Lankester 2001) and its effect on other North American deer is unknown. The second mistake was that *Parelaphostrongylus tenuis* was known to be

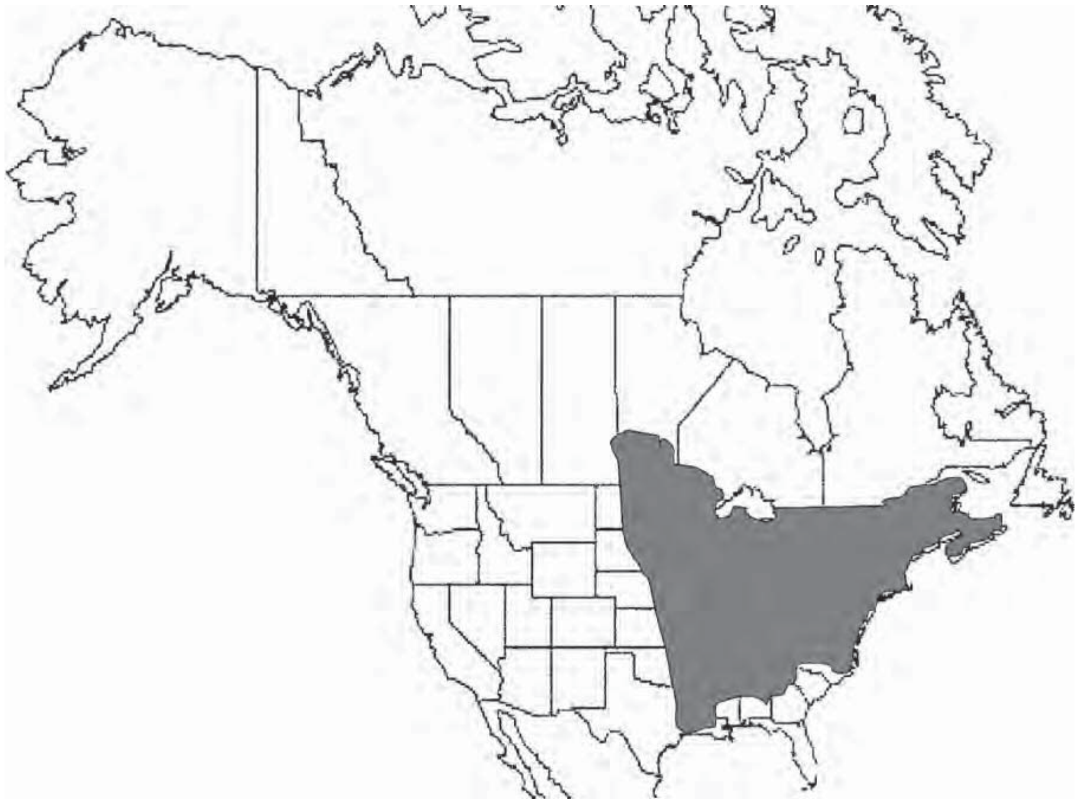

Fig. 3.4 Approximate geographic range of *Parelaphostrongylus tenuis*, the meningeal worm of white-tailed deer, in North America (adapted from Lankester 2001)

enzootic in white-tailed deer in Maine, so that introduced caribou were likely to die of this parasite, as has occurred elsewhere among transplanted caribou (Anderson and Prestwood 1981).

It also is important to define the distribution of a disease within its overall geographic range. The study of the localization of disease in nature has been termed "*landscape epidemiology*" (Pavlovsky 1966). Any departure of distribution from a random pattern should excite the interest of the investigator and suggest questions that need to be answered. For instance, botulism occurs in waterfowl across western North America but the occurrence of outbreaks is not random. Outbreaks occur repeatedly on some wetlands while adjacent wetlands, that appear similar, have no history of this disease. This observation led to a study of the occurrence of spores of the causative bacterium, *Clostridium botulinum*, in the two types of wetland. Spores were found in 59.2% of soil samples from marshes with a history of the disease, whereas only 6.2% of soil samples from marshes with no history of the disease contained spores (Wobeser et al. 1987). It is unclear whether the difference in spore density represented a cause or an effect, but the difference in prevalence of spores helps to explain the difference in occurrence of disease and it provides a basis for further questions that should be addressed.

The spatial distribution of disease events can be compared to that of an infinite number of other factors, always with a watchful eye for similarities or

differences in distribution between the other factor and disease. On a geographic scale, parameters for comparison might include factors such as the boundaries of major ecotomes, agricultural land use, proximity to lakes or oceans, density of human or animal population, altitude, climate and local weather. For example, the distribution of the nematode *Elaeophora schneideri*, a parasite of various cervids, was found to be related to altitude (Hibler and Adcock 1971). The highest prevalence occurred among mule deer in areas above 1,800 m in elevation in some regions, and in deer living between 900 and 1,200 m in other areas. With further investigation, the distribution of the disease was found to match that of certain horseflies that acted as intermediate hosts for the parasite.

On a more local scale, factors such as wind direction, sources of suspected toxin, vegetation, soil type, exposure to sunlight, water runoff, local humidity, and the distribution and local abundance of vertebrates and invertebrates might all be important in explaining the distribution of disease. Shupe et al. (1984) demonstrated a spatial relationship between the prevalence of lesions of fluoride intoxication in bison, fluoride levels in vegetation, and geothermal activity in Yellowstone National Park. Swanson et al. (1984) observed that ducklings had a very restricted distribution on highly saline wetlands in North Dakota. They found that the ducklings could only survive on these wetlands by utilizing the area around freshwater seeps. Presence of these seeps could be recognized by changes in vegetation in the immediate vicinity. Many associations that are important in understanding disease are not evident until the material is collected and compared by some mapping technique. Assistance from geographers, cartographers, meteorologists, and other specialists may be required in many studies. The process can be aided greatly through the use of computerized systems (GIS) that allow the rapid comparison of the spatial distribution of a large number of variables. Figure 3.5 illustrates the incorporation of a variety of information into the investigation of a hypothetical occurrence of fluorosis in deer. A simple spot map (a) indicates that the distribution of normal and affected deer is not uniform on the area (which should excite the curiosity of the investigator). The addition of land-use information (b) suggests that the distribution of deer is associated with forested areas but does not help to explain the clustering of affected deer. One potential source of fluoride is from certain types of industry, and fluoride may be transmitted by wind; hence, the location of putative industrial sources and wind direction information has been plotted in (c). There appears to be an association between affected deer and the industrial sources. Calculation of the prevalence of affected deer at various distances from the suspect sources would help to strengthen the association. The small cluster of cases in the north-eastern portion appears to be an independent event worthy of further investigation.

An obvious and important spatial relationship that must be investigated is that between the distribution of disease and the distribution of the population at risk. Examples of this type of association include situations where the

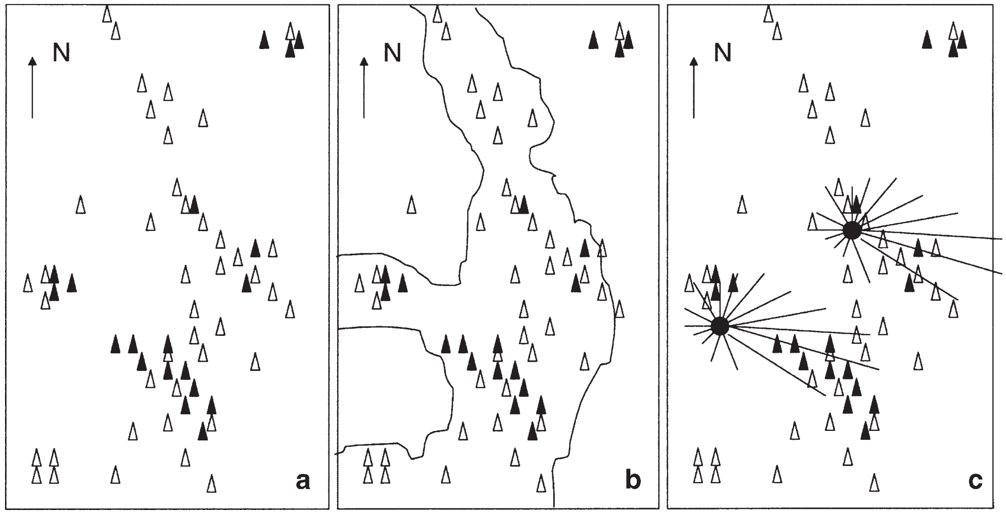

Fig. 3.5 Distribution of cases of fluorosis among hunter-killed deer (hypothetical data). The distribution of normal deer (*open triangles*) and deer with lesions of fluorosis (*filled triangles*) are shown in **a**. In **b**, the location of a forested river valley is superimposed on that of the deer and, not surprisingly, most deer were killed in wooded areas. In **c**, the location of two potential fluoride-emitting sources is indicated by *large dots*. The length of each arm of the wind rose about each of the sources is equivalent to the proportion of time that the wind blows in that direction. Most deer with fluorosis appear to be clustered about the two sources, and there appears to be some concentration of cases downwind. These associations can be examined statistically to measure their strength. The three cases in the northeastern portion of area do not appear to be associated with either of the putative sources

disease is concentrated in areas of dense population or where the distribution of disease approximates that of some segment of the population. This will be considered in Chap. 4.

3.3 Identification of the disease

An important consideration in investigating any disease is the early identification of the disease as an entity and, where possible, identification of its causative agent or factors. In many situations the causative agent cannot be identified immediately but there is a need for a working hypothesis or definition that can be used to distinguish the disease from all others, to identify new cases of the disease, and to choose the most profitable routes for further study.

In many respects, the clinician and the disease investigator face a similar problem. The clinician is presented with a sick individual while the investigator is confronted by illness in a group or population. The initial step for both is to evaluate the extent, severity, signs, and pattern of the disease. The clinician does a physical examination of the patient and then chooses the causative factor that, in her or his experience, produces disease most similar to that present in the patient. This choice is a **tentative diagnosis** and specific

treatment may be begun immediately, based on the diagnosis, or **specific** tests may be done to confirm the diagnosis. Often both are done simultaneously.

The investigator follows the same basic pattern of analyzing the information available to arrive at a **tentative hypothesis** (case definition) about the disease. Appraisal of the evidence may require involvement of individuals skilled in a variety of disciplines, and "*it should be no reflection on one's intelligence and skill to ask advice*" (Friedman 1980). High priority should be given during the early stages of an investigation to the collection and submission of a representative and adequate sample of specimens of affected animals to a well-equipped diagnostic laboratory. The purpose of this is to define the pathology of the condition, i.e., how the animals have responded to the injury or insult. On the basis of this investigation, advice, and experience, the investigator identifies the most likely cause or pathogenesis as a tentative hypothesis and then plans further investigations to answer specific questions related to the hypothesis.

The alternative to the above approach for the clinician is to do an examination and then to treat by the shotgun method (i.e., to give the patient a broad blast of every potential medication, in the hope that something will work) and/or to order all possible laboratory tests in the hope that some abnormality will be detected and provide an answer. Disease investigations are sometimes done in an equivalent manner by asking broad general questions, collecting data on every imaginable variable and applying multivariate analysis, in the hope that some correlation will be detected that will provide a clue to the nature of the disease (as will be noted in Chap. 5, significant information may be difficult to winnow from all of the environmental 'noise' surrounding a disease).

The need for rigor in arriving at a diagnosis that can be treated by the clinician or a hypothesis that can be investigated in a specific manner should be obvious. The working hypothesis or case definition of a disease should be as precise as possible and should be updated as new information becomes available. The investigator is often faced with decisions as to whether information or specimens fit within the disease he is investigating or are part of some other condition. The working hypothesis can be used to define **criteria** or rules to simplify these decisions. For example, during an investigation of mortality in wild geese in Manitoba, our working hypothesis, based on the species involved, time of year, and location was that the deaths were likely the result of avian cholera. The criteria we established for making that diagnosis in individual birds were (i) presence of suitable gross lesions and (ii) the isolation of *Pasteurella multocida* from the tissues. These criteria dictated the methods to be used and the samples to be collected for examination. During the investigation, we found that only about 70% of the birds found dead met these criteria. Approximately two-thirds of the birds that did not fit had gunshot wounds and the remainder were found to be infected with another bacterium, *Riemerella anatipestifer*. Without criteria that forced us to examine all birds in a specific manner, the number of dead birds would have been

counted, a few would have been examined, the actual mortality to avian cholera would have been overestimated by 30%, and we would have been unaware of the occurrence of another disease (*R. anatipestifer* infection) that had not been recognized in wild geese previously.

A major part of defining any disease is identification of its cause for, as stated by Susser (1973): "*The nub of epidemiology is a concern to establish causes and effects in relation to health disorders*". Earlier I discussed the observation of various types of association, such as clustering of events in time, and the spatial relationship between occurrence of a disease and other factors. In identifying associations, the investigator should be particularly interested in those associations that represent a causal relationship. This requires distinguishing between situations where factors X and Y simply occur together in time or place and those in which they occur together because X causes Y.

This book is concerned primarily with observational rather than experimental study of disease. It is important to realize that it is more difficult to establish a causal relationship by observational means than by experimentation. In a controlled experiment, one can use two groups that differ only in their exposure to factor X. If disease Y occurs only in the group exposed to X, or if Y occurs at a rate that is significantly different among the groups, we can infer that a causal relationship exists. The results of the experiment can be confirmed by replication. In observational studies it is impossible to ensure that factors other than X may not have resulted in changes in Y, since it is not possible to ensure that the groups used for comparison are identical in all respects, except this factor. Usually there is no opportunity for replication in observational studies since change is continual. However, causal relationships are inferred through observational studies and many important decisions in human and veterinary medicine are made regularly on the basis of these inferences. The following section includes some guidelines for this process.

Susser (1973) identified two general properties that must exist in any causal relationship: **direction** and **time-sequence**. The first of these implies that a change in one variable causes a change in another variable and that the reverse is not true. In other words, there is an independent and a dependent variable. For example, we might consider the relationship between the bacterium *Pasteurella multocida* and the occurrence of hepatic necrosis in ducks. Presence of the pathogenic bacterium may determine the occurrence of hepatic necrosis but presence or absence of foci of necrosis in the liver is unlikely to influence the presence of the bacterium.

The second general property is that the causative factor must precede the effect in time. Thus, it is important to establish the temporal relationship among variables when investigating a causal relationship. In some instances, the time relationship may be easy to establish, as when many birds are found dead and injured the morning after a severe hailstorm but, as noted earlier, it may be very difficult to establish a time sequence in chronic disease. For example, in our diagnostic laboratory, we often are presented with emaciated animals that have a heavy burden of parasites. These animals present a chicken

or egg type of conundrum when trying to establish a causal relationship. It is often impossible to determine, retrospectively, which condition came first, i.e., are the animals emaciated as a result of an unusually large population of parasites or are they heavily parasitized because the parasites have flourished in an emaciated, enfeebled host (this may be an example of a so-called 'symmetrical relationship' in which there is little directionality and each variable influences the other).

There have been huge advances in the ability to detect potential causative factors and disease agents, particularly through the use of molecular techniques. This enhanced ability to detect chemicals in nanogram amounts and tiny fragments of DNA is beneficial in most situations but it may complicate establishing causation, because of the need to distinguish between exposure to a potentially harmful agent, and actual disease caused by that agent. It often is not possible to expose wild animals to the agent to determine if it causes disease, and linking the presence of the putative agent to an appropriate pathological lesion becomes very important.

Some methods that have been used in establishing causation, including Koch's postulates, were introduced in Chap. 1 and their shortcomings for this purpose were discussed [although in some instances Koch's postulates are appropriate in defining causation, e.g., Woods et al. (1999)]. A group of guidelines, based on rules or canons originally formulated by the philosopher J.S. Mill, can be used to infer causal relationships. These include:

- **method of agreement**: If a disease occurs under a variety of circumstances, in which there is only one factor in common, then that factor may be a cause. A syndrome characterized by severe and often fatal degeneration of skeletal muscle has been observed in a wide variety of mammalian and avian species in association with capture or handling. The common factor in these varied circumstances is muscle exertion by the animals during the handling process. Using the method of agreement, exertion is inferred to be a causative factor in capture myopathy.
- **method of difference**: If a disease occurs in situations that are similar in all variables, except one, to those in which the disease does not occur, that factor may be causative. This rule is the basis for experimental studies in which all factors, except the one under consideration, are kept constant among groups. The method is used commonly in observational studies, but with less assurance that all factors have been identified. For example, Ohlendorf et al. (1988) found a range of lesions in birds on certain ponds in California that were heavily contaminated with selenium, while lesions were not present in birds on similar ponds that were not contaminated. This led to an inference that selenium was related causally to the disease. The weakness of this method in observational studies is that no two ponds are exactly alike, so it is impossible to eliminate the possibility that the observed difference in disease occurrence was a result of some unknown factor present in one pond and not in the other. If the

association is consistent among many ponds of both types, confidence in the inference increases.

- **method of concomitant variation**: The disease and the putative causative factor should vary systematically with each other as, for example, in a dose-response relationship. The average body weight of adult American coots at Kesterson Reservoir in California varied inversely with the concentration of selenium in their tissues. This was used as another piece of evidence to infer a causal relationship between disease and selenium (Ohlendorf et al. 1988). It is important to realize that the relationship between variables may not be linear. Some described examples are curvilinear; others exhibit various threshold phenomena with either no effect below a certain threshold level of the independent variable or no increase in effect beyond a certain threshold.
- **method of residues**: If the variation due to known causes is removed, that remaining (the residue) must be the result of other causes. For instance, if we were investigating a problem of anemia in ducks, an initial step would be to search for known causes of anemia, such as lead poisoning and infection with *Leucocytozoon simondi* infection in the affected birds. Once the proportion of anemia attributable to these causes has been identified, a cause can be sought for the residue, i.e., the cases of anemia for which lead poisoning and *L. simondi* parasitism have been ruled out.
- **method of analogy**: Susser (1973) did not include this rule in his discussion of methods, but Martin et al. (1987) suggest that it may be used (with caution). This technique involves comparison of the disease under investigation with similar well-understood diseases. If the diseases are sufficiently similar, they may have a similar or common cause. We used this method to suggest that *Clostridium perfringens* may be involved in a form of enteritis of wild geese that is similar in many regards to necrotic enteritis caused by that bacterium in other species (Wobeser and Rainnie 1987). Use of this method, as with other forms of extrapolation, is filled with risk. A single causative factor may cause dissimilar disease in different species, and similar diseases may be caused by a variety of causative factors. This method is most useful in the early stage of an investigation when one is searching for any possible clue to the nature and cause of a disease.

These guidelines form a basic framework for examining causal relationships. If factor X is causally related to disease Y, the rate of occurrence of the disease must be different in animals exposed to X than in animals not exposed to this factor. Statistical tests, some of which will be discussed in later chapters, can be used to evaluate the probability that the observed difference might be due to sampling error. If the difference in occurrence is statistically significant, this implies that the observed difference was unlikely to have been caused by sampling error, (or to be due to chance), but it does not imply that the difference was caused by X. The difference might be the result of some other unrecognized factor(s). One cannot prove a causal relationship by statistical means, because of the inability to ensure that all other factors that might have

influenced the relationship have been identified and accounted for. However, biologists are used to making decisions and reasoning with some uncertainty and this has not hindered many important advances in medicine.

Susser (1973) suggested that the final decision regarding causal relationships must be based on the investigator's subjective judgment, using all of the available evidence. He proposed a number of criteria (some of which are similar to the rules discussed earlier), that might be used in making a qualitative judgment about the significance of an association that has been found to be statistically significant. These guidelines include:

- **time sequence**: A factor must precede the disease that it causes. Many studies are based on determination of the frequency with which disease events occur in a population and the results are expressed in the form of incidence or prevalence rates. In general, incidence rates are more useful than prevalence rates for establishing time-order relationships between factors and disease. Incidence rates measure the occurrence of new cases during a defined time period and this can be related to the occurrence of the suspected causative factor before and during the period. Prevalence rates describe the amount of disease present in the population at an instant in time, regardless of when the disease began or of when exposure to the suspected cause occurred. Prevalence rates may underestimate the occurrence of transient or short-term events and overestimate the occurrence of prolonged chronic diseases.
- **consistency**: In experimental studies, consistency is demonstrated through replication. Investigators observing natural events cannot replicate their observations but, if a relationship exists between two variables, there should be consistency of findings among similar observations. The more consistently an association is observed, the greater the confidence in the association. For example, all outbreaks of necrotizing enteritis of geese that we observed between 1983 and 1991 occurred among geese on saline wetlands. This consistent finding in outbreaks that were quite varied in other regards suggests that water salinity may have a causal association with the disease (this criterion is very similar to the method of agreement described earlier).
- **strength of association**: This describes the degree to which two variables occur in association and is usually measured by calculation of the relative risk or the odds ratio. Relative risk is the ratio of the frequency of occurrence of disease in the portion of the population exposed to the putative causal factor to the frequency of occurrence in the portion that has not been exposed. If there is no association between the variables, the relative risk will be 1; the greater the departure of the ratio from unity, the stronger the association is assumed to be (relative risk and similar methods used to measure the strength of associations will be discussed further in Chap. 8).
- **specificity of association**: This is most useful for simple and direct associations in which a causative factor is necessary to produce a specific effect, and where the causative factor produces only a single or a few effects. It is

less useful where a single agent may produce a variety of effects or several agents may produce a similar effect.
- **coherence**: A causal relationship should make biological sense and the known facts about both the suspected cause and the disease should fit within the hypothesized relationship. It is important to remember that rigid application of this criterion assumes that all new information must fit within current concepts. If a causal relationship appears to be implausible, because it does not fit within the preconceived plan of how nature operates, it should not be discarded immediately. The relationship may require a revision of current thinking to accommodate a new concept. As an example, the cause of the transmissible spongiform encephalopathies, including chronic wasting disease of deer, is now generally accepted to be a structural change in a normal protein that behaves like an infectious agent. These abnormally configured proteins or 'prions' do not have a nucleus and contain no nucleic acids (Collins et al. 2004), so that they are unlike any other infectious agent and this required a complete rethinking of how infection should be defined.

The rules and criteria discussed here are a general guide to the process and, as has been noted earlier, the process of establishing causal relationships becomes much more complex for disease with multi-factorial causation.

3.4 Avian vacuolar myelinopathy—an example of defining a disease

During the winters of 1994–95 and 1996–97, bald eagles with signs of neurologic injury were observed at DeGray Lake, Arkansas, and an estimated 30–65% of the eagles wintering there died. Despite intensive investigation and diagnostic testing no cause was identified. In the winter of 1996–97, about 5% of an estimated 8,000 American coots on DeGray Lake also were seen to have neurologic disease, and eagles were also observed eating coots. At this point all that was known was **who** (bald eagles, American coots), **where** (a single lake in Arkansas), **when** (winter) and some clinical features of the disease (**what**). A major step in defining the disease came through the detailed pathology on extensive samples of dead eagles and coots and identification of a "*striking microscopic lesion characterized by noninflammatory spongy degeneration of the white matter of the central nervous system*" (Thomas et al. 1998). On the basis of the pathologic lesions, the disease was named avian vacuolar myelinopathy (AVM), and cases of AVM could be distinguished from other diseases. The type of lesion suggested a list of potential causes, primarily toxins known to produce similar lesions, and these were eliminated through testing (Thomas et al. 1998) (this step includes use of the method of analogy and the method of residues described earlier). The initial field investigations resulted in hypotheses that the similar disease in eagles

and coots could be linked through a predator–prey relationship, or through exposure to the same aquatic environment.

In subsequent years, *who* has expanded to include a number of other wild birds, and *where* now includes several lakes in the southeastern USA. Between 1998 and 2001, Rocke et al. (2002) released wing-clipped sentinel birds (coots and mallards) on a lake where AVM occurs to establish that: (i) exposure to the cause occurs on the site where the disease occurs, i.e., the birds are not exposed elsewhere and then develop disease on a lake where they winter; (ii) the disease is sharply seasonal (November and December), and (iii) disease occurs after a brief period of exposure (5–7 days). These authors suggested that the disease is caused by a chemical substance, likely of natural origin.

The hypothesized transmission from coots to eagles through predation was confirmed by feeding tissue from affected coots to raptors (Fischer et al. 2003) and it was shown that the causative factor was contained within the coots' gastrointestinal contents (Lewis-Weis et al. 2004). The latter study, together with a study by (Birrenkott et al. 2004), established that AVM could be reproduced by feeding aquatic plant material from a lake where AVM was occurring to birds. Birds fed the same type of vegetation (*Hydrilla* spp.) from a lake where AVM did not occur did not develop AVM. This led to a suggestion that the cause is associated with *Hydrilla*, rather than being *Hydrilla* itself (Lewis-Weis et al. 2004). Rocke et al. (2005) postulated that that the causative agent of AVM might be accumulated by aquatic vegetation, or "*be associated with biotic or abiotic material on its external surfaces*". The saga of AVM may not be complete, but there is evidence that an epiphytic cyanobacterial species of the order *Stigonematales*, that covers up to 95% of leaf surface of *Hydrilla* in lakes where AVM occurs, produces a neurotoxin that causes AVM (Wilde et al. 2005). Based on the information available, AVM now can be defined quite precisely:

- Who? Certain species of herbivorous aquatic birds and raptors that prey upon them.
- Where? A number (increasing) of waterbodies in the southeastern USA.
- When? Winter (varies somewhat by site)
- What? A condition that is characterized by specific clinical features (Larsen et al. 2002) and distinct pathologic lesions (Thomas et al. 1998)
- Why? Ingestion of a toxic substance associated with epiphytic cyanobacteria that grow in abundance on some species of aquatic vegetation, with secondary poisoning of raptors that eat poisoned birds.

Investigation of AVM involved scientists from many disciplines and required both observational studies in the field and laboratory experiments to develop and test hypotheses. The process of refining and testing hypotheses will be discussed further in Chap. 8.

3.5 Summary

- Formulation of a working hypothesis or definition regarding the nature of the disease must be an early step in every investigation (this is equivalent to the tentative diagnosis used by a clinician after initial examination of a patient).
- The disease definition is a dynamic entity that is modified as information becomes available.
- A disease is defined by answering the questions who? (population parameters), where? (spatial distribution), when? (temporal distribution), what? (clinical, pathologic and analytic features,), and why? (cause and pathogenesis).
- All events related to a disease should be considered in relation to time, with particular attention to clustering of events, association between events, and changes over time.
- Events and features should be mapped (in three dimensions) so that the range, distribution of events within the range, and the spatial association among factors are evident.
- Space–time interactions are important.
- Identification of the causative factor(s) is useful but not necessary for defining a disease.
- Specific methods and criteria are available for inferring causal relationships.
- Criteria and questions for future study are decided on the basis of the disease definition.

4 Collecting population data

> "*Crudely put, observers go afield to seek wildlife and return to tell the statistician how many they have found. It is then the statistician's task to determine how many animals they did NOT find.*"
>
> (Ram sey et al. 1988)

Definition of the population (the individuals of a species present in a defined area at a certain time) is central to most disease investigations and is also one of the most difficult aspects of any study of wild animals. Information about the abundance of animals is needed to assess the significance of disease, to decide on the need for management, and in most cases for assessing the effectiveness of management. There are a great variety of methods for describing a population but these usually involve elaboration of a few basic questions: (i) who is present? (ii) who is at risk? (iii) who is affected? and (iv) what effect is the disease having on the population? Answering these questions involves both a qualitative evaluation, e.g., which species are present, as well as determining the number of individuals in each group or class. This chapter will not provide a list of specific techniques for estimating populations of different species, as many references are available for that purpose. Lancia et al. (2005) provide a review and a conceptual framework for considering different methods. Emphasis here will be on the types of information that may be collected and on general principles related to data collection. It is necessary to state, at the outset, that there is no single perfect technique; all existing methods for assessing populations suffer from problems and have deficiencies, but different techniques are more useful in certain situations.

4.1 Basic features

The difference between a count and an estimate has been discussed earlier; a number of other terms used in describing population data require definition. An **absolute count** or census includes all the individuals present within an area or class and, as noted earlier, absolute counts of free-ranging wildlife are very seldom possible. **Relative counts** or estimates are used to detect changes in relation to some baseline and changes usually are reported in terms of a

proportion above or below the baseline. For example, if 400 deer were counted during a survey of an area prior to a disease outbreak, and 220 deer were seen during an identical survey after the outbreak, the relative change is a 45% decrease from the pre-disease baseline. In this example, neither the absolute number of deer on the area nor the number that disappeared is known, only a relative change has been observed.

Accuracy (or validity) is a measure of how closely the observed value corresponds to the actual state of affairs. If 200 ducks were released on a pond and 194 were counted during a survey done immediately after release, and before any population change had occurred, the survey method is correct to an accuracy of 6, or 3%. Because of the difficulty in determining absolute population numbers, the accuracy of most methods used in wildlife work is unknown. **Precision** (or reliability) is a measure of how closely a series of repeated measurements of the same thing match each other. For instance, if the same group of 200 ducks was surveyed by two different techniques, each of which was repeated five times, one might obtain the following counts:

	1	2	3	4	5	Average
Method A	180	156	227	197	210	194
Method B	196	192	189	199	194	194

The average estimate of the population size obtained with the two methods is the same, so the average accuracy of the two methods is equivalent but the precision is markedly different. The precision of an estimate is usually indicated by a measure of dispersion, such as the standard error of the mean, or one might calculate the 95% confidence limits (= ±1.96 X standard error if the estimator is distributed normally). Another way of comparing the amount of variation in the two samples is to calculate the coefficient of variation, which is the standard deviation expressed as a percentage of the mean (standard deviation X 100/mean). The 95% confidence interval for method A would include values from approximately 170 to 218, whereas that with method B would include values from approximately 191 to 197. Thus, the chances are that 19 of 20 estimates of this population made with method A will fall between 170 and 218 when the actual population is 200. The coefficient of variation of the two methods is 14.1 and 2.2%, respectively.

Estimates of population size should always contain an indication of the precision of the technique. In some circumstances, estimates may be highly precise but still be inaccurate, e.g., when a technique underestimates or overestimates the actual population by a fixed proportion. Such data, although inaccurate, still may be useful for detecting relative differences between areas or changes over time so long as the method is applied consistently. It is important to evaluate old data that may be available in planning new monitoring programs. Regular monitoring data can be combined with older sporadic data to estimate mortality rates and population growth, if the two types

of data are compatible. This was done to estimate the impact of morbillivirus outbreaks on harbour seals in England (Thompson et al. 2005).

4.2 Choosing a method

Techniques for collecting population information are chosen on the basis of the type of information required, how much information is needed, how much can be afforded, and if the results need to be comparable to those obtained by others or only to one's own data. The first choice that one must make is to decide if information is needed that describes the population as it exists at the instant (size, density, composition) or if information is required to understand changes in the population over time. If the latter is the case, it will be necessary to collect information on the four fundamental variables that result in changes in population size: natality, mortality, emigration, and immigration. Information of the first type may be sufficient during investigation of a short-term outbreak, while a more detailed study of the epizootiology of a disease will require the collection of both types of information. In many instances, it is more important to measure the **density** and the distribution of the population than to determine the total number of animals. Density is usually expressed as animals/unit of area but, in some circumstances, it may be more meaningful to express density in terms of some ecologic unit or resource, particularly if the unit is a limiting factor for the population. For instance, the number of deer using each waterhole in a xeric area may be more important for understanding disease transmission than is the number of deer/100 km^2. Measures of density are often used as indicators of population size in disease studies, e.g., Wandeler et al. (1974) used the number of foxes killed by hunting, accidents and disease/km^2 as an index of fox population size during studies of rabies. Measures of distribution and density will be considered later in this chapter.

Population estimates are usually used for comparison with other estimates. The ability to distinguish among groups and to recognize change is directly related to the precision of the method used. Precise methods are required to recognize small changes or differences. To illustrate this point, we can return to the example used earlier. Two weeks after placing 200 ducks on the pond, the number of birds present was estimated again using both methods A and B. The methods yielded identical estimates of 174 birds. With method A, this estimate is still comfortably within the 95% confidence interval (170–218) established when the actual population was 200. However, with the more precise method, B, the current estimate is well outside the confidence limits (191–197) and one should suspect that the population size had declined. The important point is that the more precise method allowed us to detect a probable population change, while any change that may have occurred was masked by the lack of precision in method A. By repeating the survey several times and calculating a mean and standard error, the estimates could be compared statistically

with the initial estimate. Unfortunately, estimates with high precision often are expensive to obtain because of the extra time and effort required to collect a large number of observations. Consequently, many of the techniques currently in use in wildlife studies have such low precision that only major changes in population can be detected.

4.3 Basic methods for determining animal numbers

Most techniques for estimating animal numbers consist of two steps: (i) **data collection**, which involves detection of the animals or some index to their abundance and (ii) **calculation** of population size. Lancia et al. (2005) identified two basic problems in any attempt to estimate animal abundance. The first relates to the probability of detecting animals that are present on the area. Most methods available do not detect all of the animals that are actually present, i.e., the probability of detection is <1. Calculation of population size usually involves some form of mathematical manipulation to account for the fact that only a proportion of animals in the population were detected and a major effort in developing population estimation methods has been in estimating the probability of detection under different circumstances. If all the individuals in a population can be counted directly, e.g., 27 cormorants on an island, the second step is then unnecessary. However, one should be aware of the problems inherent in making absolute counts, even of large birds on small islands (e.g., Haila and Kuusela 1982). The second basic problem relates to sampling. Because resources are usually limited, it often is impossible to survey the entire area occupied by a population and only a sample of the area can be examined. The dilemma lies in selecting samples that are representative and permit inference to the entire area. The choice of the appropriate method for data collection depends upon knowledge of the biology of the species and the particular situation, and many methods are available for data collection. In contrast, relatively few methods are available for calculation using the data. A critical point is that no statistical procedure or calculation will make poorly collected data into good data, nor will it allow data collected under differing conditions and circumstances to be comparable. The latter point is particularly important if data are to be compared with information collected by other investigators.

The value of replication in studies of population size can not be overemphasized. "*Unreplicated studies can lead to generalizations and unrestrained speculations; even one replication of a sample in a comparable habitat type should put some limitations on how the results are interpreted*" (Call 1986).

Methods for determining population size are based on two general assumptions: (i) that the population is stable during the period of data collection, i.e., that changes due to births, immigration, emigration and deaths are negligible, and (ii) that all members of the population have an equal probability of being

Table 4.1 Examples of the use of indices of animal abundance in studies related to disease

Species	Disease	Index
Brushtail possum	Bovine tuberculosis	Trap-catch index, fecal pellet counts[1]
House finch	*Mycoplasma* infection	Birds observed/hour[2]
Bank voles	Hantavirus infection	No. captured/100 trap nights[3]
European hare	Multiple factors	Annual hunter kill[4]
White-tailed deer	Tick infestation	Fecal pellet counts[5]
Harbour seal	Morbillivirus infection	Animals seen on haul out sites[6]

[1] Caley et al. (1999), Anonymous (2004)
[2] Based on North American Christmas Bird Count, Hochachka and Dhondt (2000)
[3] Olsson et al. (2003)
[4] Fickel et al. (2005)
[5] Rand et al. (2003)
[6] Thompson et al. (2005)

counted. Neither of these assumptions is likely to be completely valid during most studies. Problems related to the first can be minimized by keeping the data collection period as short as possible, and correction factors can be developed to correct for differences in countability among members or groups within the population.

Lancia et al. (2005) divided all techniques available into indices and population estimation methods. Indices do not actually estimate animal abundance, instead they measure some feature believed to be correlated with abundance. Examples of indices that have been used in the study of disease are shown in Table 4.1. An underlying assumption is that the relationship between the index and abundance remains constant under varying conditions, but this usually is untested. Lancia et al. (2005) caution against the use of indices, unless this assumption can be verified.

The second group of techniques is those designed to actually measure the abundance of animals. I have chosen to intermix indices and methods for estimating abundance in the following discussion.

The basic techniques for determining either an index to abundance or to measure population size consist of:

I. Counts:
  – of animals
    (a) total count
    (b) count of a sample
  – of some index of animal abundance
    (a) total count
    (b) count of a sample
II. Estimates based on removal or capture
III. Estimates based on mark and recapture

4.3.1 Population estimates based on counts

The simplest way to measure a population is to count the animals or to count some index to their abundance directly. For example, during a study of avian cholera among lesser snow geese on a lake, the entire lake could be photographed from the air and then the number of live and dead geese could be counted on the resulting photograph. Alternatively, some index such as tracks or feces, which is more easily counted than the animals, might also be used. The assumption with indices is that the abundance of the index object is directly proportional to that of the animal. Assume that we are interested in determining the population of muskrats in a marsh. It is difficult to count the animals directly due to their secretive habits; however, the number of muskrat houses might be counted from the air. It would then be necessary to determine the relationship between the number of houses and the number of muskrats. We might live-trap muskrats from a sample of houses and establish that, on average, muskrat houses in this marsh contain 2.6 muskrats with a standard error = 0.3. The estimated number of muskrats in the marsh could be calculated to be 2.6 times the number of houses, and the 95% confidence limits of the estimate would be that number $\pm 1.96 \times 0.3$. The relationship between abundance of the index and abundance of the animal must be determined for the specific area and circumstance under investigation. Extrapolation from other situations is very risky. For example, in a nearby marsh, the average muskrat house might contain 4.1 muskrats and the number of muskrats per house is likely to vary from season to season and year to year in a single marsh.

As noted earlier, a major problem with direct counts is that the proportion of animals or index objects present but not counted often is unknown. For instance, some of the snow geese in the population mentioned earlier may have been away from the lake, feeding in fields at the time of the photograph. Similarly, some muskrat houses may have been obscured by vegetation and missed during the aerial count. This type of problem can be reduced in some situations through replicate counts, e.g., by taking photographs of the geese at several times during the day and calculating the average population; or by having more than one observer count the animals on an aerial survey so that the probability of detection can be estimated (e.g., Potvin and Breton 2005). Correction factors can be developed to reduce this source of error. One could do an intensive ground search of a portion of the marsh and then compare the number of muskrat houses known to be present on the basis of the ground search to that observed from the air. The process of using ground searches to validate aerial observations is called 'ground-truthing'. The importance of ground-truthing is evident in a controversy about the use of aerial surveys for defining areas used by prairie dogs (Miller et al. 2005; White et al. 2005). Lancia et al. (2005) provide many references to methods to deal with detection probability.

If comparisons are to be made between areas, or between different time periods, the method of measurement must be consistent. Thus, a count of

snow geese made in late afternoon on a lake would not be comparable to a count made in early morning on the same or another lake, nor would counts of muskrat houses made from aircraft flying at different altitudes be comparable without some form of correction. As an example, Short and Hone (1988) found that approximately twice as many kangaroos were seen in an area during aerial surveys done at sunrise as were seen during surveys of the same area done 3 h later in the morning.

There has been very little effort directed toward assessing the efficacy and accuracy of methods used for collecting population information in relation to disease in wild animals. Even in outbreak situations, the only information that usually is available is an estimate of the number of animals found dead. Many such estimates are, in reality, only guesses. Direct body counts often have been used to calculate total mortality during the investigation of epizootics. However, the number of animals found dead, or of carcasses picked up during an outbreak, provides, at best, a *minimum* estimate of the actual number that died. The proportion of dead animals that were not found is usually unknown but may be very large. For instance, 'beach surveys' have resulted in the recovery of from 10 to 59% of marked dead seabirds of various species placed in the ocean to simulate losses during an oil spill (Beer 1968; Coulson et al. 1968; Hope Jones et al. 1970; Bibby and Lloyd 1977). Swenson (1979) estimated that a maximum of 27% of dead mule deer were found during a survey following an epizootic. Only 6% of duck carcasses placed 30 min earlier were found during a search for dead birds in a Texas marsh (Stutzenbaker et al. 1986) and we found that a line of searchers spaced 4 m apart detected only 62% of sparrow-sized models of dead birds in ungrazed pasture (Philibert et al. 1993). Fredrick et al. (1993) found that only 33–50% of dead heron chicks were detected during transects of a colony. The density of human observers in the area can have a marked effect on the probability of detection of sick or dead animals, e.g., Ward et al. (2006) placed marked crow decoys in different locations to simulate birds that might have died of West Nile virus infection. About twice as many of the birds were detected in an urban area compared to a rural area. Mark-recapture methods, described later in this chapter, are useful for estimating the proportion of dead animals that are found during surveys. Using a mark-recapture technique, we found that only about one-third of the duck carcasses present in a marsh during a botulism outbreak were collected during clean-up operations (Cliplef and Wobeser 1993). Madrigal et al. (1996) proposed a method for estimating bird mortality from pesticides. They used success in finding marked carcasses intentionally placed on the area to calculate a correction factor for birds not detected during searches.

A single count of sick or dead animals during an outbreak can only be used to calculate an estimate of the prevalence of the disease, i.e., the number affected at the time of the search. It cannot be used to estimate the total mortality. Table 4.2 shows the relationship between carcass disappearance and the number of dead animals that might be detected in searches on various

Table 4.2 Relationship between the number of carcasses detected during searches done on various days and the cumulative mortality during a disease outbreak. One hundred animals died on each of days 1 through 8, 50% of carcass disappeared /day, and 30% of the carcasses present were detected

		No. of carcasses present on each day									
Day	No. that died on day	1	2	3	4	5	6	7	8	9	10
1	100	100	50	25	13	6	3	1			
2	100		100	50	25	13	6	3	1		
3	100			100	50	25	13	6	3	1	
4	100				100	50	25	13	6	3	1
5	100					100	50	25	13	6	3
6	100						100	50	25	13	6
7	100							100	50	25	13
8	100								100	50	25
9	0									50	25
10											25
Cumulative mortality		100	200	300	400	500	600	700	800	800	800
No. of carcasses present		100	150	175	188	194	197	198	198	148	98
No. of carcasses found		30	45	53	56	58	59	59	59	44	29

days during a hypothetical outbreak. In this example, 100 animals died on each of the days 1 through 8, and mortality then ceased. It is assumed that carcasses disappeared at a constant rate of 50%/day, i.e., that the average half-life of a carcass was 1 day and that all carcass disappearance occurred overnight, and that 30% of the carcasses present were detected by the search method used. In real life, neither the rate of carcass disappearance nor the efficiency of searching is constant from day to day; however, the rate of disappearance used here is probably not unrealistic for passerine birds (Wobeser and Wobeser 1992) and the carcass recovery rate of 30% is similar to that which we have found in carcass cleanups during botulism outbreaks. It is evident from this hypothetical model that the number of carcasses recovered on any one occasion is a poor indicator of the total cumulative mortality. This becomes increasingly so as the outbreak continues over time (Fig. 4.1). In most outbreaks, the investigator does not know exactly when the outbreak began, so it is unclear where the disease is on the time scale shown in Fig. 4.1, which further complicates any attempt to extrapolate from a single carcass count to an estimate of total mortality.

Some years ago, we were interested in the extent of mortality of geese caused by avian cholera in a large area of western Saskatchewan. Our budget allowed

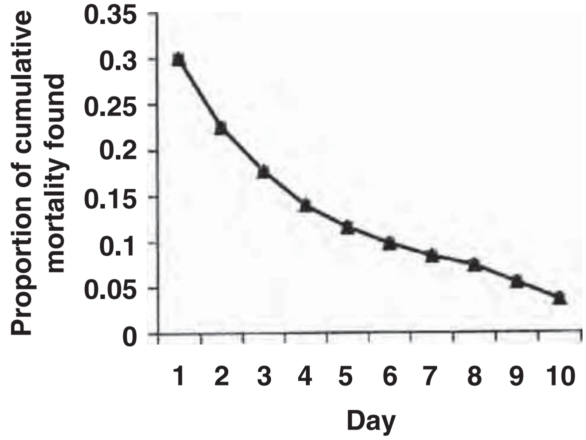

Fig. 4.1 Proportion of the cumulative mortality (all animals that died) that would be found on each day during a hypothetical die-off in which 100 animals died on each of days 1 through 8 and in which 50% of carcasses disappeared each day. The search method used detected 30% of carcasses present (data from Table 4.1)

one aerial survey of the area per week. Two questions arose in the planning stage of this work. Our first concern was related to the proportion of dead geese present in the area that would be detected from the air, i.e., how good was our search technique? The second related to the length of time that individual carcasses persisted in a recognizable form in the field. We needed an answer to the first question to understand the accuracy of the technique. This was determined by a process of ground-truthing in which we counted the number of carcasses present in marshes using ground searches and then compared these counts to counts made the same day from the air (this double sampling, using two different techniques, provided a measure of the probability of detection during the aerial surveys). We desired an answer to the second question to determine if there would be carry-over from one week to the next, i.e., were we likely to count the same carcasses on successive weeks and, hence, overestimate the incidence of disease. We marked recently dead goose carcasses with inconspicuous tags, left the birds in situ, and observed them daily until they disappeared. More than 50% of carcasses were gone within 4 days and all disappeared within 6 days. Combining the results of these two trials, we felt that our method provided a reasonably accurate count of the carcasses present and that we could be confident that few or no carcasses would persist from one weekly survey to the next. However, many birds that died between surveys would be missed because they had been removed, so that our estimate of the number of dead birds was conservative. This type of information might be used to derive a mathematical model that would allow estimation of total mortality on the basis of repeated surveys but, to my knowledge, this has not been done.

In most disease studies, a complete count of the population is impossible, and some form of sampling is necessary. A complete count might be done on a portion of the area, and then the population on the total area calculated

based on the assumption that the density of animals on the sampled area is representative of that in the total area: N (total population)/ A (total area) = n (number in sampled area)/ a (sampled area), in which case, N = An/a. Each individual count is a sample and must be supplemented by additional counts, (either repeated counts on the same area, or counts of several areas), so that an estimate of the population, together with confidence limits of the estimate, can be calculated. The type, size, shape, and number of sample plots that are used are based on knowledge of the biology of the species and methods available to the investigator. Often, sample plots are geographic areas but they may also be some ecologic unit, such as a tree or den-site. Sample plots may be of various shapes and each of circular, square and rectangular plots has particular advantages and limitations.

The line transect method is widely used for estimating density and abundance of wild animals (Buckland et al. 2001). It is appropriate for use during the study of disease but has received little attention. In a line transect, the observer moves along a randomly chosen straight line within the area, counting all the animals that are seen, and measuring either the perpendicular distance from the line to where the animal was seen or the distance to the animal and the sighting angle. It is assumed that not all animals are detected and that the probability of detection decreases with distance from the line. This probability can be calculated and this allows the density of animals to be estimated. We studied the line transect method for estimating the density of dead passerine birds in two habitat types and found it to be reasonably accurate, providing that the search line was sufficiently long so that at least 40 birds were located (Philibert et al. 1993). In a pasture with grass from 30 to 70 cm tall, search lines 1.6 to 4 km long were required to find sufficient birds when the known density was 50 birds/ha. Rivera-Milán et al. (2004) conducted field trials of line transect using chicken carcasses to establish the usefulness of this techniques for assessing pesticide-induced mortality of wild birds in Argentina. We used line transect to estimate density of nests and bird carcasses during a study of the role that Franklin's gulls play in waterfowl botulism (Soos and Wobeser 2006).

The location of sample plots or transect lines should be based on knowledge of the distribution of individuals within the area and it is a serious mistake to assume that the distribution of animals will be random or uniform. Dispersion may result from environmental factors, such as the availability of suitable habitat, or from behavioral factors, such as gregariousness or territoriality. Often a pilot study using random sampling on an area to determine the distribution of animals is necessary. Three general patterns of distribution are shown in Fig. 4.2. When the population is dispersed in a random or regular distribution, unrestricted or simple random sampling may be adequate. In this method, the area is divided into suitable sized plots by means of a grid and plots are selected randomly for sampling. This method meets the general requirement for random sampling, in that each plot has the same probability of being included as every other plot. When the population is found to be

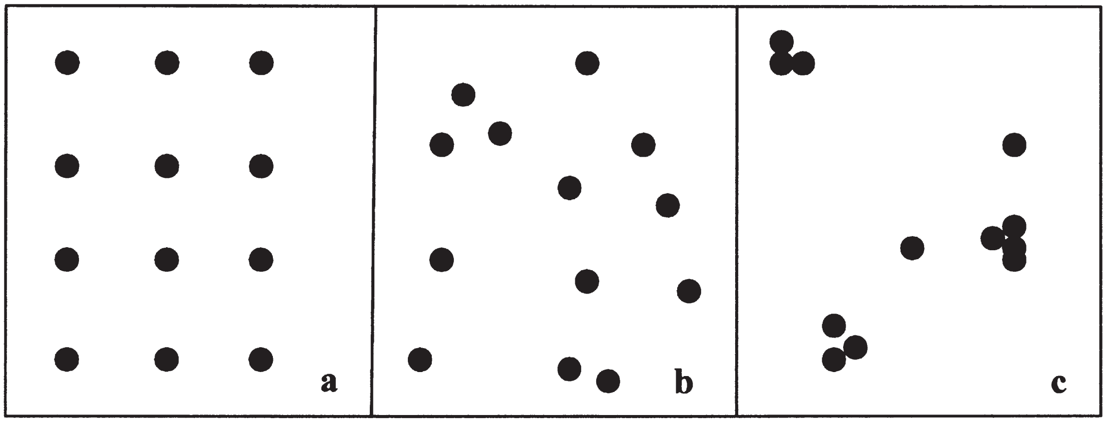

Fig. 4.2 Examples of three types of distribution of animals within an area: **a** Regular; **b** Random; **c** Aggregated

distributed in an aggregated or clumped manner, there may be advantages in using **stratified** random sampling. Many types of stratified sampling have been described, and the reader is referred to Davis (1982) for specific examples. The basic technique consists of dividing the area into sub-areas, often based on the density of animals in these **strata,** and then sampling within the strata in a random fashion. A major advantage of stratified sampling is one of efficiency, in that the sampling effort can be concentrated in the strata that contain most of the population. Whitlock and Eberhardt (1956) provide an early example of the use of stratified sampling for finding deer carcasses during a disease study.

The choice of the appropriate number of samples that should be collected in any survey is an important decision because collection of excessive samples is wasteful and an inadequate sample size may limit confidence in the estimate. The appropriate sample size is determined by the size of the difference one wishes to detect. As noted earlier, greater precision (and a larger number of samples) are required to detect small as compared to large changes in the population. Methods for determining minimal sample size under various conditions will be discussed in Chap. 7. Davis (1982) includes several examples of the use of various techniques for determining sample size in studies of population size. The choice of an appropriate sample size is not an easy matter, and assistance should be sought from a knowledgeable biometrician whenever possible.

4.3.2 Population estimates obtained by removal or capture of animals

These methods have not been used extensively in disease studies but may be appropriate in certain circumstances, particularly for evaluating the effectiveness of some types of disease management. The simplest method of this type is to calculate an index of animal abundance by measuring the number of animals captured relative to catch effort. This system has been used in many studies of

small mammals. For example, the number of meadow voles captured/1,000 trap nights, (a trap night is one trap set for one night) provides an index to the number of voles present, and this can be used to compare the relative abundance of animals in an area at different times or to compare the density in different areas, providing that the same trapping method is used in all instances and that the probability of detection remains constant at different levels of population. Rosatte et al. (1986b) measured the effectiveness of skunk population reduction for control of rabies in Alberta by comparing the number of skunks caught per unit of catch effort at various stages of the program. A standardized trap-catch index is used to assess the effect of brushtail possum control in New Zealand (Anonymous 2004; Coleman et al. 2006).

When animals are removed from a population and the removal operation is repeated again and again, the number of animals caught during each successive trapping period should decrease. The progressive decrease in the number caught can be used in a variety of ways to estimate the original population. The assumptions for these methods are that each animal in the population is equally likely to be caught, that the probability of capture does not change during the removal process, that the population is closed (no increase or loss except through capture), and that the number caught is proportional to the number on the area. Two simple graphical methods for using this type of data are shown in Fig. 4.3. The graphs might depict the number of skunks captured each week during a hypothetical trapping campaign to control rabies in an area. Obviously, home range and activity of the animals, length of the removal period, and immigration into the area, will have a great effect on this technique. The assumptions listed above are seldom completely valid in real life. For more details of this type of procedure and the related mathematical methods for calculation, see Lancia et al. (2005). As an alternative to actually removing animals from the area, captured animals may be marked and released. Marked individuals are then treated as though they were not present (although they make traps unavailable to capture new animals, so that the number of trap-nights must be reduced for calculations). An advantage of this method over removal is that habitat is not left empty on the study area, reducing the likelihood of immigration of new animals from outside the area (Bracher et al. 1986).

Another method uses the change in ratio of occurrence of some feature or index of the population, as a result of removal of animals, to estimate population size. Swenson (1979) used the change in the proportion of bucks in a deer population, as a result of the hunting season, to estimate the population in an area before an epizootic. Prior to the hunting season, 18% of the deer observed on the area were males, while after the hunting season males comprised only 9% of the deer seen. About 44 bucks were known to have been killed on the area by hunters. The change in proportion of males from 18% (S_1) to 9% (S_2) was assumed to be the result of removal of these 44 (n) animals. If N is the population of males on the area prior to the hunting season, then: $S_1–S_2/n = S_1/N$ or $18–9/ 44 = 18/ N$ and $N = 88$. If there were 88 males on the area prior to the hunting season, the total population $= 88/18 \times 100 = 488$.

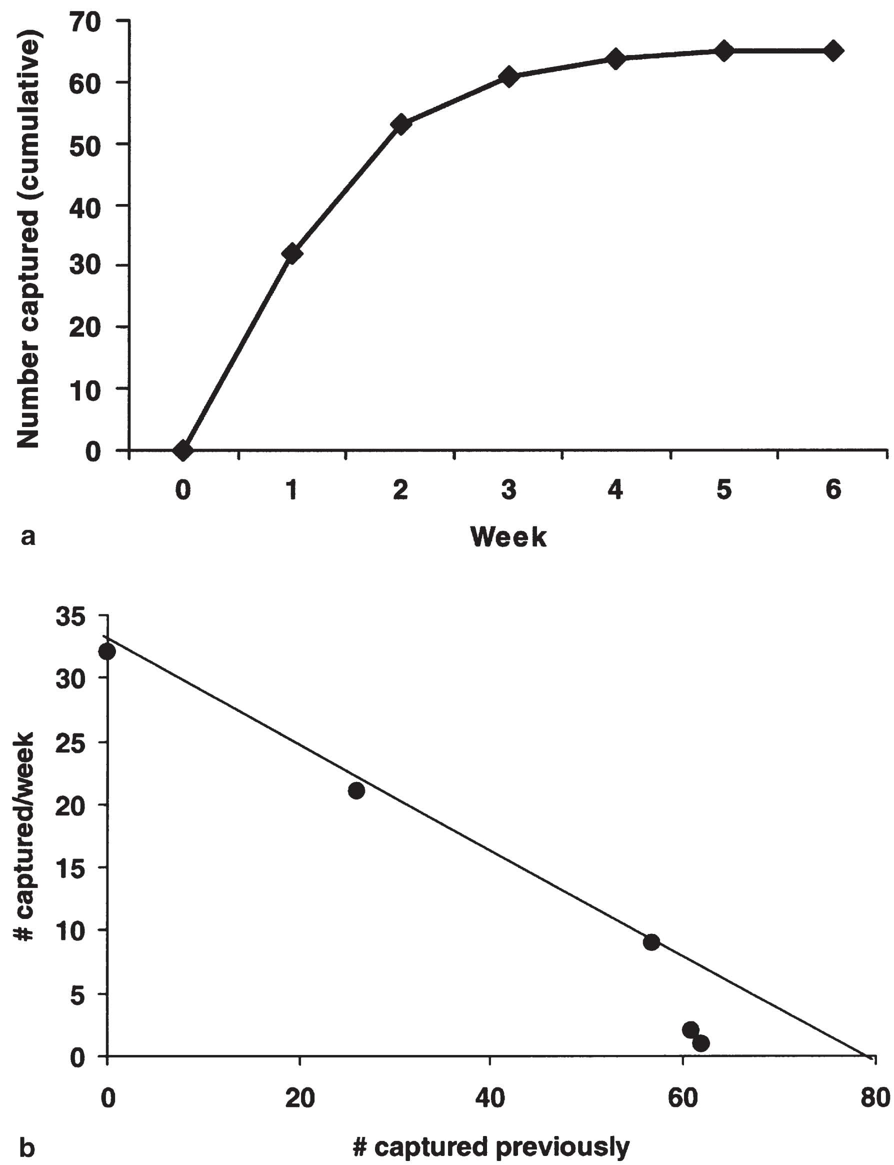

Fig. 4.3 Two simple graphical methods for using trapping data to estimate population size. In both cases, the animals captured were removed from the population. In **a**, the cumulative number of animals captured is plotted and the total population size is estimated by the asymptotic point on the resulting line. In **b**, the number captured in each time interval is plotted against the number captured previously with the total population being estimated by the intercept of the resulting line with the x-axis

Similar calculations can be done using track counts or other indices measured before and after a period of depopulation, such as in the skunk control program shown in Fig. 4.3.

A basic and serious problem with this method is that each of the values used in the calculations (e.g., the proportion of bucks in the population) is an estimate with an error component. When such estimates are used in calculations that involve division or subtraction, the compound error increases dramatically. The error component of the final estimate (population size in this example) might easily be ± 100% of the estimate. Lancia et al. (2005) should be consulted for other assumptions required for this technique.

4.3.3 Estimation of population based on mark-recapture

Estimation of population size based on recapture of marked individuals is one of the most widely used techniques in wildlife work. An array of methods are available (Manley et al. 2004) but most are derived from tests (Peterson method, Lincoln index) based on the assumption that the ratio of marked to unmarked animals in a sample collected from the population is representative of the same ratio in the population: N (population size)/ M(number marked and released) = n (number in sample)/ m (marked animals in sample). Assume that 100 animals were captured, marked, and released in an area. A few days later, ten marked animals were recaptured among a sample of 40 trapped animals, then: N/100 = 40/10, and the estimated population N = 400. Mark-recapture techniques may be useful in any situation in which animals or objects can be marked and recaptured later. For example, Swenson (1979) used this technique to determine the efficiency of a search for carcasses during an epizootic in deer. We used a mark-recapture method to test the effectiveness of carcass collection during a botulism outbreak in ducks (Cliplef and Wobeser 1993). Dead ducks were marked with inconspicuous tags and replaced where they had been found in the marsh just prior to the start of a clean-up operation by other individuals. All carcasses collected were then examined for tags prior to disposal. In one trial, 103 dead ducks were marked. Of the 85 carcasses collected during cleanup of the area, 20 had been tagged. The carcass collection was only about 19.4% effective (20 of 103 marked carcasses were collected). Using the formula N/M = n/m and solving for N, the estimated number of carcasses present in the area was 438. The actual number of carcasses actually present was likely even greater, since some dead birds were undoubtedly missed during both the initial search when we marked carcasses, and the carcass collection.

If conspicuous marks are used, or if the animals have distinctive natural marks, the animals may be observed visually or by other means without being captured, e.g., Bartmann et al. (1987) used radio-collars to relocate deer during a study of the accuracy of aerial surveys. Mowat and Strobeck (2000) used mark-recapture analysis based on DNA recovered from hair samples collected in "*hair-catchers*" to estimate abundance in a population of grizzly bears. Many elaborate methods for dealing with mark-recapture information are available (see Manley et al. 2004), with the Jolly-Seber model (Jolly 1965; Seber 1965) being most important. The same basic assumptions are required in all these methods: (i) the marks are not lost during the study period, (ii) there is no addition to the population during the study, (iii) the marking process does not affect subsequent survival of the animal (i.e., mortality is the same for marked and unmarked animals), and (iv) marked and unmarked animals have the same probability of being captured.

Methods have been developed for testing how well data fulfill some of these assumptions (Davis and Winstead 1980) and techniques are available to deal with variable probability of capture (Rexstad and Burnham 1991); however,

mark-recapture techniques often have been used without regard to these assumptions, or to the limitations of the methods. Investigators should be particularly concerned that the capturing and marking process does not, in itself, act as a morbidity or mortality factor. If marked animals develop capture myopathy or suffer other injury during capture and handling, estimates of population size, and survival rate based on the recapture of these individuals will be biased and not representative of the actual population (Höfle et al. 2004; Abbot et al. 2005). In general, a large proportion of a small population must be marked to obtain reliable estimates and this may nullify the advantages of these techniques (Bartmann et al. 1987). Manley et al. (2004) and Lancia et al. (2005) should be consulted for further discussion of mark-recapture methods.

4.4 Population distribution

The distribution of animals within an area is a fundamental feature of a population but "*it is a feature that is extremely difficult to describe in precise and meaningful terms*" (Clark and Evans 1954). As noted earlier, it is foolhardy to assume that any population of animals is distributed randomly across the landscape. Some species maintain and defend territories, while others share or have extensive overlap among adjacent home ranges. The spread of infectious diseases geographically is influenced by the degree of overlap and interaction between neighbors. Aggregated or clumped distributions create special problems for measuring animal abundance and often are extremely important in understanding the ecology of both infectious and non-infectious diseases. For example, Wright and Gompper (2005) describe the effect of a clumped distribution on parasites of raccoons. One technique for quantifying spatial relationships is by use of nearest neighbor analysis (Clark and Evans 1954). In this analysis, the expected average distance from an individual to its nearest neighbor in a randomly distributed population can be calculated based on the number of animals and the size of the area. This then serves as a basis for comparison with the average measured distance between individuals and their nearest neighbor in the population under consideration. Nearest neighbor analysis also can be used to compare groups, such as infected and uninfected individuals, as in studies of tuberculosis in badgers and cattle in Ireland (Olea-Popelka et al. 2005) and the United Kingdom (Woodroffe et al. 2005). In both of these studies, spatial clustering of animals with tuberculosis was detected. Woodroffe et al. (2005) found that infection with *Mycobacterium bovis* was clustered spatially at a scale of 1–2 km in both badgers and cattle, which has obvious implications for management.

The distribution of animals may change at different times of the year and this may be important in disease transmission if, for example, the rate of contact is higher between infectious and susceptible individuals when they are aggregated. Animals also may be aggregated artificially, enhancing disease

transmission, as is thought to be important in transmission of tuberculosis among white-tailed deer aggregated by artificial feeding in Michigan (Miller et al. 2003) and in transmission of brucellosis among elk concentrated on feeding grounds (Thorne et al. 1982).

A feature of animal distribution that is important for understanding disease is dispersal of animals, since this may explain in part how disease moves across the landscape. Dispersal has been defined as the movement an animal makes from its point of origin to the place where it reproduces (Caughley 1977). Dispersal is difficult to detect or measure. The traditional method has been to use mark-recapture, and particularly radiotelemetry, to follow individual animals. For instance, in studies of tuberculosis in wild elk near Riding Mountain National Park, Manitoba, most marked individuals stayed close to the original site at which they were marked but a few individuals dispersed across many kilometers of open farm land to another area of suitable elk habitat, so that sampling for tuberculosis had to be extended into this area. Buechner (1987) defined dispersal in terms of the number of territories, home ranges, or units of area capable of supporting a resident animal that are crossed by a dispersing individual. This is a useful concept for considering dispersal in terms of disease because it provides an image of the number of resident animals with which a dispersing animal is likely to have contact. In a study of tuberculosis in ferrets in New Zealand, Caley and Morriss (2001) found that very few juveniles dispersed, i.e., left the home range where they were born. The distance that animals move may be influenced by habitat conditions, such as vegetation conditions, and animals living in areas of poor or patchy habitat may move greater distances and contact more conspecifics than animal in uniformly good habitat (Root et al. 1999). Disease may also alter the distance that animals move or disperse, e.g., the average distance moved by non-rabid raccoons during a study in New Jersey was 1.5 ± 0.5 km, while rabid raccoons moved an average of 8.4 ± 4.3 km (Roscoe et al. 1998).

Another method for estimating dispersal (and immigration) in a population is through use of molecular techniques to identify the population structure by examining the genetic profile of individual animals (Waser and Strobeck 1998). Through the use of assignment tests, the natal population of individuals can be identified and the proportion of immigrants can be estimated more rapidly and with less fieldwork than is required for mark-recapture studies (Berry et al. 2004). This was used to characterize feral pig populations in Australia and allowed assessment of the efficacy of population control, identification of groups that acted as source for reinvasion after population control, and delineation of reinvasion corridors along river courses (Hampton et al. 2004). Immigration may confound interpretation of a local disease event. Baker et al. (2001) found increased genetic diversity in bank voles from contaminated sites at Chernobyl but could not determine if this resulted from increased mutation because of radiation or from increased immigration into the contaminated site because of higher mortality there.

4.5 Vital statistics

Changes in population size and density occur because of variations in the rate of entry of animals into the population through birth or immigration and in the rate of loss of animals through death or emigration. The methods discussed to this point have been concerned only with the abundance of animals and do not provide information on vital statistics, such as sex and age ratio, natality, recruitment, survival, and mortality, which may be as important as the number of animals for understanding a disease. For instance, Mills et al. (1999) found that the apparent prevalence of antibody to hantavirus in a population of wild rodents was not proportional to population density. This seems counter-intuitive but could be explained when sex and age composition of the population over time was known. When the population was increasing, the prevalence of animals with antibodies to hantavirus was low, because the population was being diluted continuously by the addition of young animals that had not yet become infected. When environmental conditions were less favourable, reproduction declined and the population decreased, and the population consisted largely of older animals that had been infected and had antibodies. In some animals, it may be very difficult to detect an effect of disease on the population without considering the sex/age structure. For example, seabird populations are made up of many overlapping generations and the population contains a pool of non-breeding birds. Losses, such as might occur from an oil spill in which an entire age cohort dies, may not be obvious because of recruitment from the pool of non-breeders, as well as other forms of compensation (Burger and Gochfeld 2002). Vital statistics related to the population are calculated by observation of samples of living animals, or examination of samples of dead animals that have been collected, harvested, or found dead. Dinsmore and Johnson (2005) provide a very thorough review of methods for collection and analysis of this type of population data.

The samples used must be representative of the population and, for this to be true, each animal in the population must have an equal opportunity to be identified and sampled. Most samples of wild animals are biased in some way and, as a general rule, one should treat all samples as biased until proven otherwise. It is better to assume a biased sample and to search for causes of bias (so that they can be measured and reduced early in the study) than to assume that sampling is free of bias, only to discover later that the data are flawed. Samples collected by observing free-ranging animals may be biased by differential behavior, activity, distribution or visibility of the various sex and age groups. This variation may change diurnally or seasonally, e.g., brightly hued, singing male songbirds are much more conspicuous than are their mates during the breeding season, but this bias may be less severe at other times of year. Connolly (1981) felt that counts of mule deer conducted during the summer underestimated the number of males in the population because males moved less than females at this time of year. Counts in late autumn were thought to reflect the population composition more accurately than those done in the summer.

It is difficult (or impossible) to distinguish the sex and age of many species at a distance and some type of trapping or capture may be necessary. Samples collected by trapping or other means of capture are usually biased. Juvenile animals may be unusually susceptible to capture because of naivety, males may have an increased likelihood of encountering a trap because of larger home range size, and social dominance may determine which animals enter the trap first (Garrott and White 1982). Even mass-capture techniques such as cannon-netting or drive-trapping of waterfowl may not produce random samples from the population (Raveling 1966; Sulzbach and Cooke 1978; but see Morez et al. 2000).

Animals killed by hunters are a common source of samples for disease studies. Such samples may be biased not only by differences in vulnerability of animals to hunting but also by conscious or subconscious selection by the hunter (Coe et al. 1980; McCracken et al. 2000). Animals dead of other causes, for instance road-kills, may also be used, but disease investigators (if anyone!) should be aware that most mortality factors affect each sex and age group at a different rate and that such samples are often not representative of the population. During carcass collections, conspicuous species are likely to be found at a proportionately greater rate than are cryptic species (Linz et al. 1991; Philibert et al. 1993; Cliplef and Wobeser 1993).

There is no single method for avoiding bias and obtaining representative samples. Techniques should be chosen on the basis of a thorough knowledge of the biology and behavior of the species being studied, and of the local area. The advice of experienced field biologists is particularly valuable in this regard. It is a sound principle to examine and compare samples collected in more than one way from the population, whenever it is possible to do so. For example, assume that we are studying the impact of a disease on a deer herd. We find that there is a small proportion of fawns among a sample of deer killed by hunters. This might be the result of a low proportion of fawns in the population, perhaps because of disease, or it might be because of some other factor such as active selection against fawns by hunters. Evidence of the age composition of deer harvested in the same area in earlier years, and in the same year in adjacent deer herds would be helpful for interpretation, if such data are available. One could also be more confident that the proportion of fawns in the population was actually reduced if few fawns were seen during an aerial survey of the area and if there was also a paucity of fawns among a sample of road-killed deer. In this instance, all the data sources would be corroborative. Connor et al. (2000) described a method for detecting bias in data from hunter-killed animals. Bias may have little effect if the same technique is used repeatedly to measure relative changes over time or between areas, so long as all samples are biased in a similar manner.

4.5.1 Sex ratio

Knowledge of the gender composition of the population is needed for the calculation of other vital statistics, many of which differ between the sexes, and it is necessary for understanding the reproductive potential of the population. Sex is

an important intrinsic determinant of disease and many diseases are distinctly sex-oriented. These include diseases that are: (i) related to structures or functions that occur only in one sex, such as mastitis and uterine infections in the female, and reduction of lipid soluble PCBs and other chlorinated hydrocarbon residues in females as a result of lactation (Addison and Brodie 1977), (ii) related to sexually oriented activities, such as the occurrence of brain abscesses in male deer as a result of injuries suffered during the rut, (iii) transmitted venereally, as well as diseases such as brucellosis in which the major impact is on the reproductive organs. As an example, young male bison are particularly prone to contract brucellosis because they are particularly interested in materials associated with the birth process that are the major route of transmission (Rhyan 2000). Many other diseases occur more commonly in one sex than the other, although the reasons for this are unclear. For example, many male white-tailed deer have some degree of degenerative joint disease by the time they reach 5 years of age, while this condition is uncommon in females of any age (Wobeser and Runge 1975a). Males of some species of game birds are better able to withstand cold and starvation than are females, while the reverse is true in other species (Latham 1947). A striking example of a sex-associated disease is the synchronous mortality of the entire male segment of the population that occurs annually in the dasyurid marsupial *Antechinus stuartii* (Barker et al. 1978).

During a disease outbreak, it often is possible to determine sex-specific numerators by counting and determining the sex of affected and dead individuals. However, such counts may be biased by differences in visibility between the sexes, e.g., male birds usually are more conspicuous than females, or because of differential expression of the disease in the two sexes. It is more difficult to obtain suitable sex-specific population denominators needed to calculate rates. This is particularly true for inconspicuous species that lack obvious sexual dimorphism. It is important to remember that an unequal sex distribution is normal within some animal populations.

The proportion of each sex in the entire population is the general sex ratio; age-specific sex ratios also may be calculated. The sex ratio traditionally is expressed as the number of males per 100 females (e.g., 114 males:100 females) but there may be advantages in expressing it as a proportion (males = 0.53, females = 0.47) if the ratio is to be used in other calculations.

4.5.2 Age composition

Information on the age distribution within a population is needed to describe a disease, for calculating other ratios, and also may provide important information on the history of the population and its response to disease. Age is an important determinant of disease and many diseases are distinctly age-associated. Some diseases occur only in the very young, e.g., myiasis (infection by fly larvae) caused by the fly *Wohlfahrtia vigil* is limited to nestlings (Craine and Boonstra 1986). This parasite, and the mortality it causes, would be completely overlooked unless this age group is examined. Many infectious

diseases occur at the greatest prevalence among young animals, in some cases because older animals in the population have protective immunity acquired as a result of infection when they were younger. Other diseases, such as rabbit hemorrhagic disease, are found predominantly in older animals. This may be because of transient protective immunity acquired from the young from the dam, susceptibility associated with the aging process (many degenerative diseases and neoplasia appear to be of this type), cumulative exposure (certain long-lived parasites and many cumulative toxins), or because the disease is slow to develop and only becomes evident in older individuals. As an example of the latter situation, macroscopic cysts of the protozoan parasite *Sarcocystis rileyi* are not found in hatch-year ducks during fall migration because the parasite requires at least 5 months development in the duck before cysts are visible to the naked eye (Cawthorn et al. 1981). Anderson and May (1985) present evidence that in many diseases of humans there also may be age-related changes in the rate of infection of susceptible individuals. It is probable that similar phenomena exist among wild animals.

The ease with which observers can differentiate among age groups varies among species. In birds, it often is only possible to distinguish between hatch-year and adults although, in some species, sub-adults that have not bred but are more than 1 year old also may be distinguishable. The actual age of many mammals can be determined by examining the replacement of deciduous teeth in young animals and by the presence of cementum annuli in permanent teeth of adults. However, cementum annuli may be unreliable in some situations (McCullough 1996). In the field, differentiation between young-of-the-year and adults may be all that is possible. Depending on the method used for counting, there may be serious bias because of differences in visibility of one age or sex group. For example, aerial surveys gave a good estimate of the total number of adult bison in a group, but the number of calves was underestimated markedly (Wolfe and Kimball 1989).

The young/adult ratio is important in most disease studies because it is a measure of reproductive and rearing success. In the investigation of certain diseases, it may be necessary to measure this ratio at several times during the year because different disease mechanisms act at different stages of life. Consider a moose population in which the calf/cow ratio has been noted to be very low during surveys done in the autumn of successive years. Further sampling at several times of year might reveal a variety of different scenarios, each of which suggests mechanisms that should be investigated:

Calf/Cow ratio

Mid-gestation	Post-partum	Autumn	Potential mechanisms
Low	Low	Low	– poor breeding success, low conception rate, early fetal death
High	Low	Low	– late abortion, stillbirth, high perinatal mortality
High	High	Low	– high mortality of calves after perinatal period

The last of these scenarios was found in a moose population in Saskatchewan in which the loss of calves was attributed to predation. It also was found to be the situation in certain bighorn sheep bands in Colorado where lambs were dying as a result of transplacentally transmitted lungworm infections that caused severe pneumonia in mid-summer when the nematodes matured (Woodard et al. 1974; Schmidt et al. 1979).

When suitable information is available, it may be useful to construct an **age pyramid** (Fig. 4.4). Such information must be interpreted with care, but it may provide evidence of the past history of the population, particularly if pyramids for a succession of years can be compared. In Fig. 4.4, population A has a high reproductive rate, indicated by the large number of young, a relatively high rate of mortality of animals in their first year (assuming that the yearling population was similar to that of the current young), and then a lower rate of mortality among older age groups. This general pattern is thought to be normal for many wild animal populations. Population C appears to have had an extremely low reproduction or survival of young for the past 3 years and, based on the sample, it appears that recruitment into the herd has been very low. This was the type of pattern seen in bighorn sheep herds that suffered successive years of high mortality of lambs from mid-summer pneumonia. Population B appears to have experienced 1 year of poor reproduction and/or survival so that one age class or cohort is almost absent from the population. This is the type of pattern observed in arctic-nesting birds as a result of a year with unfavorable nesting conditions.

Information on the average age at which individuals become infected, age-specific prevalence of infection and immunity, as well as the population age structure and average life expectancy, is critical for understanding the population biology of any disease. The most common method for collecting this type of data is through cross-sectional surveys, and serologic surveys in particular, in which the occurrence of various factors can be related to age. Figure 4.5 illustrates the proportion of animals of various ages that have experienced a disease, based on the prevalence of antibodies to the agent. The average age at which infection occurs in the population can be estimated from this type of data, and this statistic can be used to estimate other values, such as R_0 the basic reproductive rate of the disease (this subject will be discussed in Chaps. 10 and 13). Studies by Van Rensburg et al. (1987) and Harris and Smith (1987) provide excellent examples of the use of age-related information of this type in the study of the impact of a disease, and of a control program, respectively, on the demography of wild populations.

4.5.3 Measures of reproduction

Knowledge of the reproductive ability and success of a population is essential for any understanding of the population ecology of a disease. This information is needed to define the effects of disease on the population, for predicting

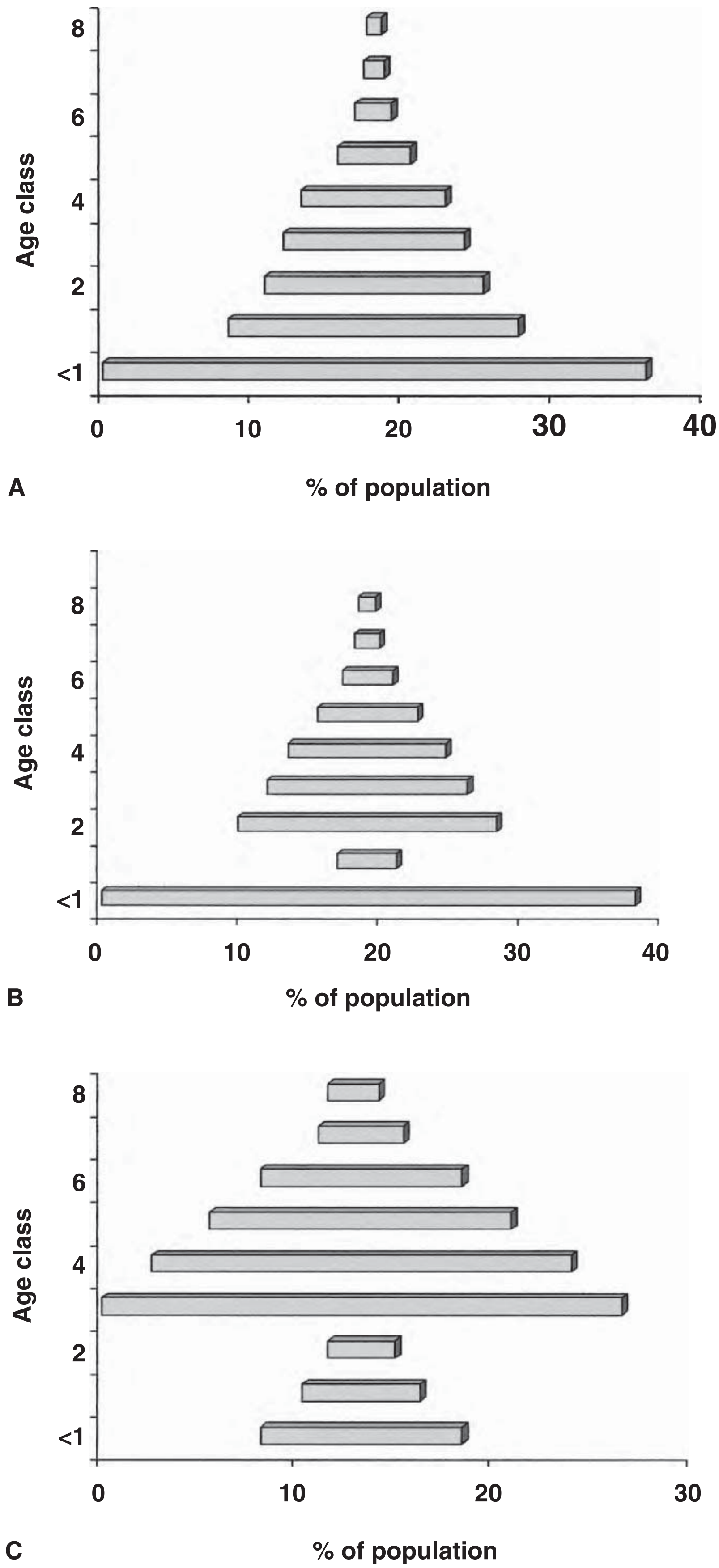

Fig. 4.4 Age-pyramids for three hypothetical populations of wild animals. The differences in pattern among the populations are discussed in the text

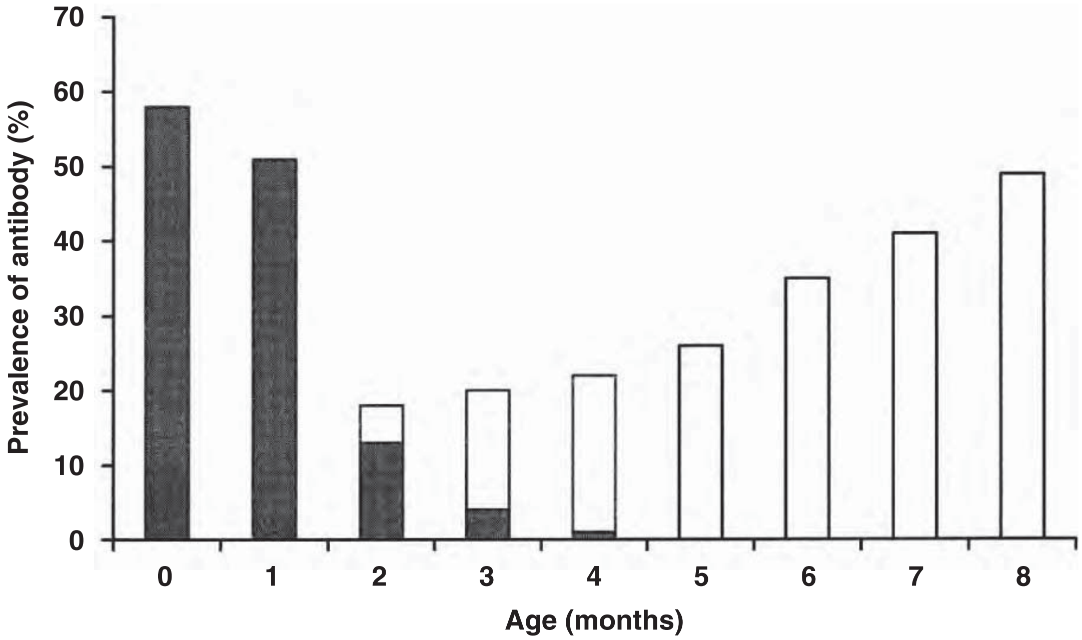

Fig. 4.5 Age-specific prevalence of antibody to disease agent X in a population. Many neonatal animals had antibody acquired passively from the dam (indicated by the *filled portion of the bars*). The prevalence of this type of antibody waned rapidly during the first 3 months and the subsequent increase in antibody prevalence was the result of exposure to agent X. This actively acquired antibody is indicated by the *open portion of the bars*

the response of the population to a disease, and for designing and assessing the effect of a management program. Studies by Wandeler et al. (1974) and Bogel et al. (1974) provide an insight to the importance of the reproductive biology of a host (the fox) in the epizootiology of a disease (rabies), and the impact of a high reproductive rate on the success of attempted control procedures.

Fecundity is the term used to describe the potential reproductive output of a species. Fertility is the actual reproductive performance of the population and is usually expressed as a rate. A number of terms have been used for this rate, including reproductive rate, birth rate, and natality rate. Each of these is a ratio of the number of live offspring produced during some period to some measure of the population during that period. Unfortunately, the term 'offspring' is interpreted arbitrarily, depending on the stage of development that is measured. It might mean the number of fertilized zygotes, the number of implanted embryos or eggs laid, the number of young born alive, or the number of young that hatch. Each of these is a valid measurement and each may have some particular significance for an individual disease but the researcher must take care to define the rate used. The most commonly used numerator in natality rates is the number of young born or hatched alive. In human populations the annual birth rate is expressed in relation to a denominator of the average number of persons alive in the population during the year. For reasons

discussed earlier, this statistic is seldom used for wild animals, and most natality rates are expressed in relation to the adult female (fetuses/pregnant female, fawns/adult doe, ducklings/adult hen). If the number and proportion of adult females in the population are known, more general rates can be calculated.

Measurement of reproductive rates is done by sampling the population and is subject to all the biases discussed earlier. Calculations are done in the same manner as for other population variables and, because the sample size usually is small, the precision of the resulting estimates is often poor. Until recently, measurement of reproductive success prior to parturition (during pregnancy) involved post-mortem examination of the reproductive tract. Application of techniques, developed for use in domestic animals, such as field laparoscopy (Zwank 1981), ultra-sound examination (Smith and Lindzey 1982), analysis of blood hormone (Seal and Plotka 1983) and pregnancy-specific protein B (Noyes et al. 1997; Russell et al. 1998), rectal palpation in large species (Follis and Spillet 1974) and measurement of fecal steroid metabolites (Schoenecker et al. 2004) allow the researcher to follow individual pregnancies and to measure in utero reproductive loss. However, there must always be concern that capture and handling, necessary to examine the animals, may affect their reproductive performance adversely. Studies such as that by DelGiudice et al. (1986) to determine the impact of immobilization on pregnant deer are needed to validate data resulting from these techniques. In some species, examination of the uterus for placental scars, the ovaries for corpora lutea, the mammary glands for milk, or the plumage for the presence of a brood patch may allow retrospective assessment of the recent reproductive history of an individual female. The number of young seen with adults or the age ratio in samples of harvested animals can be used as an index of fertility. Another number, the recruitment rate, i.e., the number of young, particularly of females, that reach reproductive age and, hence, are recruited into the productive segment of the population is often very important in understanding the impact of disease at the population level.

As noted in Chap. 2, a difference between investigating disease in wild animals and investigating disease in humans and domestic animals, is the need to consider the impact of disease on life-time reproductive success or fitness. This is extremely difficult, except in small populations that can be followed intimately over many years, as has been done with red-billed choughs (Reid et al. 2003), or through the use of extensive radio-marking as has been done with caribou (Adams and Dale 1998).

4.5.4 Mortality and survival

Although mortality is a stock-in-trade of the disease investigator, the term is seldom used in its population sense in papers dealing with disease in wild animals. In contrast, wildlife managers use the concept regularly. The **mortality rate** is a measure of the probability of death occurring during a prescribed

interval of time, and is defined by the equation: mortality rate = number of deaths during period/number alive at beginning of period. It is important to note that the mortality rate applies only to those individuals alive at the beginning of the period. This is in contrast to the **death rate**, which appears in the literature occasionally and may be confused with mortality rate. The death rate equals the number of deaths during period/average number in population during period. Death and mortality rates are equal if the time period under consideration is instantaneous, or if additions to the populations match the number of deaths exactly but, in most instances, the rates are different. Death rate will not be considered further here.

A third rate, **survival,** is used widely and is the reciprocal of mortality, i.e., survival = 1 – mortality, and is defined by the formula: survival rate = number alive at end of period/number alive at beginning of period. As with mortality, the survival rate refers only to the individuals alive at the beginning of the period.

Information on the death of deer during winter taken from Potvin et al. (1981) illustrates these rates. During the winter of 1974, an estimated 100 deer died from a population of about 480. The mortality rate over the winter was 100/480=0.21 and the survival rate was 380/480 = 0.79.

Survival rates for consecutive periods may be multiplied to calculate a cumulative survival rate. If the survival rate for a group of birds in April, May, and June was 0.89, 0.92, and 0.89, respectively, the overall survival rate during the 3-month spring period is the product of these, or 0.73. If any two of the population at the beginning of a period, the population at the end of a period, or the number of deaths are known, mortality and survival rates can be calculated.

What is measured in most studies is the apparent survival rate rather than the true survival rate, because fidelity to the area is usually not measured. If animals leave the area permanently (emigrate), the apparent survival will be biased low relative to the true survival. Return rate to the nesting colony in the following year has been used to measure the effect of parasite treatment (Hannsen et al. 2003) and immunization (Hannsen et al. 2004) on annual survival of female common eiders. It was believed that apparent survival was very similar to true survival in these situations because fidelity to the colony was known to be strong.

Studies of survival/mortality usually involve marking and releasing animals. The assumption is that the capture and marking process has no effect on survival. That this is not a safe assumption is illustrated by the examples in Table 4.2; however, other studies have not detected an effect of the system used for marking on survival (Swenson et al. 1999; Esler et al. 2000; Conway and Garcia 2005; DelGiudice et al. 2005; Powell et al. 2005). Whenever possible, a marking system should only be used when its potential effect on the results has been assessed. Radiotelemetry has been used extensively for direct measurement of mortality rates in wild animals and is particularly useful for studying neonatal or cryptic animals that are hard to find. This technique has the advantage that animals can be located for necropsy shortly after death if motion-sensitive transmitters (mortality switches) are used. The results obtained

from radio-marked individuals may be very different from those animals found by other means. For instance, in a study of mortality among reintroduced Eurasian lynx, 72 dead lynx were examined of which 15 were found because they were radio-marked. In the entire group, 18% died of infectious disease, while 40% of the radio-marked individuals died of infections (Schmidt-Posthaus et al. 2002). The survival rate of offspring has been measured by placing a radio on the mother, so that the group can be located for observation (Duncan 1986; Eberhardt et al. 1989). Evelsizer (2002) used radiotelemetry to compare the survival of ducks during botulism outbreaks on wetlands where carcasses were collected to that of ducks on wetlands with no carcass cleanup. Even if animals can only be relocated occasionally, the data collected may be useful, e.g., Ringelman and Longcore (1983) used a technique for estimating average survival time of ducks that were located infrequently.

A number of techniques have been developed for calculating mortality rates mathematically. Many of these were derived from methods developed in entomology or fisheries and only simple examples will be presented here.

Catch:effort: It often is easier to measure some index to the population than to determine population numbers, as indicated earlier. Changes in catch:effort can be used to calculate mortality, provided that all the assumptions mentioned previously in this chapter are valid. For example, during a study of long-tailed weasels, an average of 8.7 animals was captured/1,000 trap nights in the autumn, while only 4.3 were trapped/1,000 trap nights in the spring. The estimated mortality rate over the winter (during which no additions occurred as a result of births) = 8.7–4.3/8.7 = 0.51, and the survival rate = 4.3/8.7 = 0.49 (one assumption in this example is that weasels are equally susceptible to capture in autumn and spring, which may or may not be true).

Mark-recapture: A number of techniques are available for estimating mortality or survival using mark-recapture information. If animals are marked at one time and then recaptured on two occasions subsequently, a modification of the catch:effort method can be used to measure mortality in the interval between the two captures. If animals can be recaptured repeatedly, the survival rate can be estimated by plotting the proportion of the marked animals known to be alive against time (Getz 1970). The hypothetical data set in Table 4.3 illustrates information from a population of 12 marked animals in which recapture was attempted at monthly intervals. Paradis et al. (1993) used capture/recapture information in a model to estimate sex and age-related survival in a small rodent population. Newman et al. (2002) used mark-recapture to compare the survival of foxes affected by sarcoptic mange to that of uninfected foxes. Infected foxes survived only about one-fifth as long as uninfected foxes.

Another method called the "*triple catch trellis*" by Ricker (1958) requires two mark-and-release operations with different marks applied at each time, and one recapture. If 120 muskrats (M_1) were captured, marked and released in autumn and an additional 60 (M_2) were captured and marked and released early the following spring, the proportion of each, ($R_1 = 30$, $R_2 = 25$), captured during a later trapping period could be used to estimate over-winter survival: survival = $R_1M_2/(R_2 + 1)(M_1) = 30 \times 60/(25 + 1)(120) = 0.58$ (Table 4.4).

Table 4.3 Examples of studies that have detected negative effects of capture/marking on subsequent survival of animals

Species	Handling or marking procedure	Effect
Canada goose	Neck bands	Reduced survival[1]
Mallard	Radio transmitter	Reduced survival[2]
Wild turkey	Radio transmitter	Negative effect on wing growth[3]
Grey partridge	Radio transmitter	Adverse effect on survival, reproduction and body mass in some years[4]
Northern pintail	Radio transmitter	Reduced body mass[5]
Cassin's auklets	Radio transmitter	Reduced growth of chicks from radio-marked adults[6]
Emperor goose	Neck collar, radio transmitter	Reduced survival, breeding, clutch size[7]
Blue-winged teal	Radio transmitter	Altered behavior[8]

[1]Castelli and Trost (1996), [2]Paquette et al. (1997), [3]Hubbard et al. (1998), [4]Bro et al. (1999), [5]Fleskes (2003), [6]Ackerman et al. (2004), [7]Schmutz and Morse (2000), [8]Garretson et al. (2000)

Table 4.4 Example of using capture-recapture information to estimate the survival rate of a group of animals by using the proportion known to be alive at various times during the study. The animals were marked in December and an attempt was made to recapture each animal at monthly intervals

Animal	January	February	March	April	May	June
1	R[a]					
2						
3	R					
4						
5	A[b]	R				
6	A	A	A	R		
7						
8	A	A	R			
9	R					
10	A	A	A	R	A	R
11	R	R				
12	A	A	R	A	R	
Proportion alive	.75	.50	.33	.25	.17	.08

[a] R – recaptured
[b] A – assumed to be alive because recaptured later

For derivation of this formula and variance calculation, see Ricker (1958) and Seber (1973). Bird-banding analyses are derived from this general principle but have become very sophisticated (see Brownie et al. 1985) but a huge number of birds need to be banded to estimate survival with precision, because of the low rate of recovery (Sheaffer and Malecki 1995).

Change-in-ratio: Changes in the proportion of some ratio, usually sex or age, during a period of mortality can be used to estimate mortality. This technique is used extensively to estimate mortality as a result of hunting and deserves consideration for use in disease outbreaks. The general requirements are that the population contains two groups that can be readily distinguished, e.g., males-females, young-adults, or two species and that, during the period of mortality, one of the groups is removed at a higher rate than the other. The proportion removed from the entire population (i.e., the overall mortality rate) is defined by the formula: mortality rate $=P-R/R-K$, where P is the proportion of one group within the population prior to the removal, R is the proportion of the same group in the population after removal, and K is the proportion of the group among those removed. A hypothetical avian cholera epizootic will be used to demonstrate how this method might be used. Prior to the outbreak, the ratio of snow geese:white-fronted geese in the area was 30:70 (P=.30). The ratio among a large sample of dead birds collected during the outbreak was 50:50 (K=.50), and the observed ratio following the outbreak was 10:90 (R=.10). Assuming that all losses were due to the disease and that no birds moved into or out of the area during the period, the proportion of the total population that died (the general mortality rate) $= .30 - .10/.10 - .50 = 0.50$. The species-specific mortality rate can be calculated by multiplying the general mortality rate by the appropriate K/P value: thus, the mortality rate for snow geese $= 0.50 \times .50/.30 = 0.83$ and for white-fronted geese $= 0.50 \times .50/.70 = 0.36$.

The technique obviously works best in situations in which the groups can be distinguished at a distance in the field. The ratios observed must be representative of the true situation and, if the ratios are similar, small biases or errors in any ratio will affect the estimated mortality greatly (Davis and Winstead 1980). Dinsmore and Johnson (2005) suggest that because the assumptions required for this method are stringent, these should be considered carefully before the method is used.

Life tables (mortality-survival tables): The methods described above have been concerned with general mortality and survival rates of the population. In some circumstances it may be necessary to know the age-specific mortality or survival rate. The concept of a life table has been developed for the study of age-specific mortality and longevity in human populations. A life table presents the history of a group of individuals or cohort born simultaneously (usually in 1 year) by tabulating the number surviving at the end of each interval (often a year) until the last individual is dead. Construction of such a table for a human population requires relatively few assumptions because records are kept of all deaths and the total population is measured at regular

intervals by census. In contrast, those working with wild populations usually have incomplete population data and must make many assumptions and inferences in the construction of a life table (Davis and Winstead 1980). The techniques may be useful in long-term studies where information is available over a period of years but attempts to estimate age-specific rates from a single census or sample of a population taken at one time require that the population have a stationary age distribution, and such estimates are plagued by problems of sampling variability (Polacheck 1985). Those interested in these techniques should consult Caughley (1966, 1977) as well as Lancia et al. (2005).

4.5.5 Cause-specific rates and special ratios

Much of the information in this chapter has dealt with general rates (mortality, death, and survival). The disease investigator usually is interested in cause-specific rates, i.e., as a result of a single disease. The same general principles and techniques are used for collecting such information; however, care must be taken to ensure that both the numerators and denominators used are appropriate. A common mistake during the investigation of outbreaks of disease in wild animals is to assume that all of the individuals found dead succumbed to a single factor. It should be obvious that animals are dying continuously of a number of conditions and that these non-specific deaths continue to occur, even in the midst of a catastrophic epizootic. Whenever possible, a large sample of individuals should be examined in a diagnostic laboratory to determine the proportional mortality rate for each cause of death, i.e. the number of deaths attributable to each cause/total number of deaths. This rate can then be used to adjust the numerator.

The appropriate denominator for general rates is the total population but some individuals within the population may not be at risk of developing a particular disease because of age, sex, prior exposure, or other factors. Cause-specific rates should be calculated using only the segment of the population that is at risk as a denominator. This may require additional sampling to determine the proportion of the population that has identifiable resistance. A hypothetical outbreak of canine distemper in raccoons may illustrate these points. The number of raccoons that died in a county was estimated to be 300, and the total population in the area prior to the outbreak was estimated to be 800. A sample of 40 raccoons found dead was submitted to a diagnostic laboratory and, of these, 28 (70%) were found to have died of canine distemper, while the other 12 died of a variety of other causes. Serum collected from a sample of raccoons captured in the area shortly before the outbreak was available in a serum bank. Of these animals, 65% had antibody to canine distemper at a titre considered to be protective. Thus, only about 35% of the population was actually at risk of developing canine distemper. The general mortality rate during the epizootic was $300/800 = 0.38$, while the cause-specific rate for canine distemper among the animals at risk was: $(300 \times .70)/800 \times .35 = 0.75$.

4.6 Summary

- Wild animals seldom can be counted directly and most population parameters must be estimated.
- Accuracy is a measure of how closely an estimated value corresponds to the actual value. Most estimates of wild populations are of unknown accuracy.
- Precision is a measure of the extent to which repeated measurements of a single population agree with their mean. Population estimates should include an indication of their precision.
- Methods for determining animal numbers consist of two steps: (i) detecting the animals (or some index to their abundance) for counting, and (ii) using the number detected to estimate population size. The second step involves mathematical manipulations to account for the proportion of the population that is not detected. Measurement of the probability of detection should be a part of all studies.
- Most methods for measuring animal abundance assume that the population is stable during the data collection period and that all members of the population have an equal probability of being detected. Neither of these assumptions is totally valid in most measures of wild populations.
- Animal abundance may be estimated by: (i) using counts of animals or of some index to their abundance, (ii) measuring changes that occur when a known number of animals are removed, or (iii) measuring the proportion of previously marked animals that can be recovered or observed.
- Additional methods are required to collect life history information, such as sex and age ratios, reproductive performance, mortality and survival rates, needed to understand the population effects of disease.
- Most samples of wild animals are biased in some way. The effects of techniques, such as animal capture and marking, on the factors being measured should always be assessed.
- There is no perfect technique for collecting information on animal abundance; various techniques have advantages under some circumstances.
- Calculation of cause-specific information is necessary to separate the relative effects of different disease factors.

5 Defining environmental factors

> "*We believe that providing a set of rigid rules for inventorying and monitoring wildlife habitat would be presumptuous since nature itself is complex, diverse and dynamic.*"
>
> (Cooperrider et al. 1986)

Some diseases are restricted in occurrence to one or more sharply defined foci, but these represent only the most extreme examples of a general principle that each disease occurs in a particular type of environment or niche, and that most diseases have a somewhat restricted geographical distribution. The study of the localization or **nidality** (from the Latin *nidus* for nest) of diseases has been termed "*landscape epidemiology*" (Pavlovsky 1966). This discipline was developed for the study of infectious zoonotic diseases (Audy 1958) but the principles and techniques are applicable to both infectious and non-infectious diseases of wild animals.

The occurrence and localization of disease are determined by a variety of factors including some that relate to the host, some that relate to the causative agent or risk factor, and a collection of features that are neither a function of the agent nor a characteristic of the host population. The latter are considered to be 'environmental' factors. Included in this category are abiotic (non-living) features, such as climate, topography, soil and water, and biotic (living) features including flora and fauna. Humans are a part of the biotic environment but given the magnitude of transformations caused by humans and the rapidity with which these occur, human factors will be considered separately. Factors are often so intertwined that it is difficult to clearly separate the role of individual elements in the pathogenesis of a disease. For example, climate may interact with a disease through its historic effects on the formation of soils in the area, as well as through its more immediate effect on the distribution and well-being of animals, plants, disease agents, and human activities on the area.

Characterization of the environmental conditions associated with disease is an important part of every investigation; however, deciding which factors are important among all of the environmental 'noise' may be difficult. "*The relevant environmental noise to be taken into account is not a single abiotic factor, but rather the combined effect of all abiotic and biotic factors that affect birth and death rates*" (Jonzén et al. 2002). In general, one starts by defining the broad characteristics of the macroenvironment or habitat and

then works to more detailed levels of measurement as pertinent microenvironmental features are identified. For instance, during a study of an infectious disease one might find that the causative agent should not be able to persist in the general area, because of unfavorable conditions of temperature, humidity, or insolation. It might then be necessary to examine protected microenvironments, such as within animal burrows or under the leaf litter of the forest floor, to explain survival of the disease in the region. The process of gathering information proceeds from the general and large to the specific and minute, and knowledge of microhabitat conditions is often necessary for understanding the ecology of a disease.

Environmental factors may affect disease directly, e.g., when inclement weather acts synergistically with a parasite to reduce survival, or it may act indirectly through a series or cascade of events. For instance, Jones et al. (1998) described a chain of events that related acorn production, gypsy moths, mice, and white-tailed deer to the risk of Lyme disease for humans. The environmental factor(s) and disease may occur close together in time, as when poisoning follows rapidly after a pesticide application, or there may be a considerable time delay or lag between one event and the other, e.g., the abundance of nymphal ticks infected with *Borrelia burgdorferi*, was influenced by growing conditions and the acorn crop 2 years earlier (Jones et al. 1998; Ostfeld et al. 2001, 2006).

Many of the disciplines needed for defining environmental conditions associated with disease are full-fledged sciences in their own right, each with its individual methodology and jargon. A multi-disciplinary approach is generally required for detailed investigation of environmental factors.

It is impractical to prepare a general list of all the environmental factors that should be examined during the investigation of a disease. Only certain factors will be appropriate for any individual investigation and the relative emphasis placed on different types of information must be specific to the disease. The intent here is to review the major categories of environmental information, with examples of how each is important in some specific disease conditions. The investigator starting the study of a disease might use this as a broad catalogue, from which he or she can construct their own checklist of various types of information that might be required. He or she should then decide how each type of information might relate to the particular situation with which they are dealing. Statistical techniques, such as discriminant analysis and multivariate analysis, may be very helpful in detailed studies of environmental factors and biometricians should be consulted in planning such a study. Papers by Carey (1979) and Carey et al. (1980) remain as good examples of the application of these methods in the detailed study of the environmental factors involved in a disease in a wild population. Cooperrider et al. (1986) provide an excellent general guide to techniques for assessing various environmental factors in relation to wild animals.

A difficulty in assessing the effects of environmental variables on disease is that not all individuals in any population are affected equally by environmental

conditions. For instance, in territorial species, those individuals occupying a territory have better access to resources than non-territorial individuals, so that the less privileged members are disproportionately affected in times of resource shortage. The effect of the environmental change may not be obvious if only the territorial members are monitored.

5.1 Characteristics of the physical environment

5.1.1 Topography

The surface configuration of a region affects its climate and soils, as well as the occurrence and distribution of plants, animals, water, and human activities. Turbulence caused by topographic features may also influence the vertical and horizontal dispersion of air-borne materials, including infectious agents and noxious chemicals. Narrow valleys may constrain the air flow so that variations in wind direction are largely ineffective and air-borne pollutants follow the course of the valley. In other circumstances, a ring of hills may form a catch-basin for stagnant air, pollution, and air-borne toxicants. Any of the above features may influence the occurrence of disease, although the causative mechanism may not always be evident. For example, Moro (1967) reported that anthrax in Peru was confined to livestock in areas below 2,000 m in elevation, although most of the livestock lived above that level and animals at higher altitudes were susceptible to experimental challenge. The cause for this phenomenon was not established, but possession of the information would be extremely useful in developing a management program for the disease.

Many other diseases have an altitudinal distribution. Avian malaria is an important disease of indigenous birds of Hawaii. Van Riper et al. (1986) found that *Plasmodium* sp., mosquitoes, and native birds were each distributed in a different manner along an elevational gradient. The highest prevalence of malaria occurred among birds at mid-elevational ranges, where there was the greatest overlap among *Plasmodium*, mosquitoes, and birds. In a similar manner, there is a >1,000-fold difference in transmission intensity of human malaria between lowland and highland areas of Tanzania related to vector abundance and prevalence of infection in mosquitoes (Bødker et al. 2003). It has been suggested that high-elevation habitats may act as a refuge from parasites for dark-eyed juncos (Bears 2004). In Switzerland, rabies spread among foxes along mountain valleys with the intervening alpine areas, above 1,500 m in elevation, acting as barriers to spread between valleys (Steck et al. 1982). River courses in Australia acted as corridors along which feral pigs re-established following population reduction (Hampton et al. 2004). The low prevalence of antibody titres to bluetongue and epizootic hemorrhagic disease (vector-transmitted viral diseases) in mule deer in Oregon has

been explained by separation of the mule deer, that occupy summer range at 2,100–2,700 m in elevation, from major breeding sites for the vector fly (*Culicoides* spp.) that are located at about 1,200 m in elevation (Kistner et al. 1977). Moose and white-tailed deer, although sympatric in a general area, may be separated altitudinally and this may reduce exchange of parasites, including *Parelaphostrongylus tenuis* (Telfer 1967; Kelsall and Prescott 1971). Even relatively minor topographical features may have a significant effect on a disease. Carey et al. (1980) found that both the Colorado tick fever virus activity and its tick vector were concentrated on the south-facing slopes of a montane region because of the proper combination of soil-type, soil moisture, temperature, and sunlight at that site. The prevalence of tuberculosis in brushtail possums declined with altitude and steepness of slope on a study site in New Zealand (Caley et al. 2001). Hensley (1976) reported that adult flies of *Cuterebra* spp. aggregate on sunny hill tops to mate and predicted that there should be a high prevalence of infection with this parasite in mice near such sites.

The basic method for studying topographic effects consists of mapping disease events in relation to topographic features. A word of caution is necessary here because differences in sampling in various types of terrain may lead to spurious conclusions about the distribution of disease factors. Roads (and most other human activities) tend to follow the path of least resistance, and are usually concentrated in valleys rather than on ridge-tops, so that the reporting of diseased animals may be biased in favor of areas with easy access. Care must be taken in planning studies to ensure that all topographic features are sampled in proportion to their occurrence in the area.

5.1.2 Climate

Climate is the composite or generally occurring weather conditions of a region averaged over a series of years and is distinct from weather, which is the state of the atmosphere at a definite time and place with respect to wind, temperature, cloudiness, humidity, barometric pressure, etc. Climatic measurements are usually expressed in terms of the mean of observed values over an extended period, such as the average daily maximum temperature on a date each year during the period 1970–2000. The disease investigator usually is concerned with weather conditions during the investigation of short-term phenomena, such as disease outbreaks, and with climate when determining the distribution of animals, disease agents, and human activities. The features of greatest interest are temperature, precipitation, barometric pressure, sunlight, humidity, and wind speed and direction. Meteorological data from government agencies describe the macroweather of a region and are measured with standardized instruments, e.g., in Canada, temperature is measured in a louvered wooden shelter located 1.5 m above ground, in a level grassy area, with the nearest obstacle at least four times its height away. This type of data

is readily available and may be adequate for some studies but it may not be representative of conditions on the actual site where disease is occurring. For example, Overstreet and Rehak (1982) measured a temperature of 43°C on a tern nesting area, where nestlings were dying of hyperthermia, while the official temperature reported for the general vicinity was 32°C. In many circumstances, the disease investigator will require measurements taken at ground level or in some micro-environment. A biometeorologist should be consulted for assistance in choosing suitable techniques for making such measurements.

Climatic conditions generally determine the geographic range of animals. Poikilothermic species, such as mollusks and arthropods that are vectors of many disease agents, are particularly sensitive to temperature and humidity (Mellor and Leake 2000; Gubler et al. 2001). Abnormal weather over a short or long period of time may allow populations of animals to expand their range, and also may allow diseases to occur in new areas. Extensions of a wild population beyond the normal, climatically determined range may result in mortality (e.g., Miller et al. 1972). Weather also can be a direct causative agent of disease. In temperate regions, winter is often the limiting climatic season, with low temperature, wind chill, snow depth and character, and length of winter all being important severity factors. In these areas, winter survival is often the dominant limiting factor for populations and may overshadow the impact of other forms of disease. Animals in such situations usually die in an emaciated state but the cause of death is more complex than simple starvation. The effects of various sublethal diseases, such as parasitism, may determine which individuals survive the winter, e.g., Gulland (1992). This is an instance of context-dependent virulence in which a disease agent has a severe impact on host animals that are stressed in other ways. Individual host animals may have inadequate resources for both defending themselves against the disease agent and for maintenance under severe climatic conditions (Brown et al. 2003). Verme (1968) and Crete (1976) developed indices to winter severity that can be used to predict the impact of various conditions on white-tailed deer. Similar indices could be developed to measure the effect of winter on other species. Verme (1968) provides useful insight into the importance of microclimate for animals under winter conditions. In hot, arid regions, drought during summer acts in a manner analogous to that of winter in northern latitudes (Anthony 1976).

Even brief periods of inclement weather may be a direct cause of mortality of wildlife. Examples of this include heatstroke in least tern chicks (Overstreet and Rehak 1982), mortality of deer and grey partridge as a result of winter blizzards (Richards 1975; Knapton 1980), and death of birds during hail (Johnson 1979; Priehl 1979) and thunderstorms (Kittler 1979). In some such instances it may be important and difficult to differentiate between the direct effect of weather and that of other potential causes of disease (Henny et al. 1982). As an example of the latter, I observed a situation in which carcasses of eared grebes and American coots killed during a hailstorm

became substrate for growth of toxin by *Clostridium botulinum*. Additional mortality occurred several days after the storm, because of botulism. Inclement weather also may have an impact on wild populations through effects on reproduction or on resistance to infections. For instance, spring drought on the prairies has been documented to result in reduced clutch size and nesting success of arctic-nesting snow geese (Davies and Cooke 1983) and pronghorns surviving a severe winter in Montana had very poor fawn production the following spring (Martinka 1967). Soerjadi et al. (1979) provide an example of the interaction between cold and the pathogenic bacterium *Salmonella typhimurium*.

In general, the survival of microbial agents outside of the host animal is influenced negatively by increased temperature and ultraviolet irradiation, and positively by increased humidity. This is important in many disease situations, as it may directly influence the availability of viable microorganisms for transmission and the distribution of the disease. For instance, Hansen et al. (2004) identified areas with high humidity and low temperature as sites with a high risk of infection of humans with the tapeworm *Echinococcus multilocularis*, because of better survival of parasite eggs in these locations. Environmental temperature has a marked effect on the activity of invertebrates that may be involved as disease agents, and as intermediate hosts or vectors of disease agents. For example, cold temperatures reduce the activity of larvae of the winter tick *Dermacentor albipictus* and, hence, decrease the probability of infection of moose (Drew and Samuel 1985). Similarly, the gastropod intermediate host of the meningeal worm *P. tenuis* is inactive below 14°C and, hence, is less available to cervids during cool weather (Upshall et al. 1987). Epizootics of hemorrhagic disease (bluetongue and/or epizootic hemorrhagic disease) cease abruptly with the onset of freezing temperatures in autumn (Trainer and Karstad 1971) because of the effect of temperature on the insect vector. High temperatures also may affect invertebrates. Environmental temperatures above 28°C reduce the efficiency of fleas in transmission of *Yersinia pestis* because the bacterium does not block the fleas (i.e., obstruct the digestive tract resulting in regurgitation) at high temperatures (Olsen 1981). This may influence the transmission of plague to humans (Cavanaugh and Marshall 1972). As noted earlier, the temperature within a microenvironment may be considerably different from that in the general area, and invertebrates may be able to persist under conditions that appear highly unfavorable, through their location in a sheltered microenvironment. We have found that the temperature within the microenvironment of a decaying duck carcass may be as much as 32°C warmer than the air temperature and this microclimate phenomenon may help to explain the continuation of outbreaks of avian botulism during cold weather (Wobeser and Galmut 1984).

Precipitation can influence the occurrence of disease in a variety of ways. Drought conditions may concentrate animals, including vectors, resulting in increased exposure to infectious diseases, such as necrobacillosis in deer (Rosen et al. 1951), parasites in waterfowl (Cornwell 1963), anthrax in African

game animals (Pienaar 1967), avian cholera in waterfowl (Rosen 1972), and St. Louis encephalitis virus (Shaman et al. 2002). Drought may force herbivores to consume toxic plants, because of lack of normal foodstuffs (Hayes and Shotts 1958). The incidence of botulism in British waterfowl is inversely related to the amount of rainfall (Smith 1979), although the mechanism is unclear. The distribution and prevalence of a number of parasites, including lungworms in bighorn sheep (Forrester and Littell 1976), sinus worms in British weasels (King 1977) and meningeal worms in deer (Kocan et al. 1982) have been related to rainfall. Six of nine African antelope species passed larger numbers of parasite eggs in their feces during drought years that in non-drought years; this was attributed to reduced nutritional intake and impaired resistance to parasites (Ezenwa 2004). Epizootics of Rift Valley fever in domestic animals in Africa are associated with periods of heavy rainfall that flood areas used as habitat by the vector mosquitoes. Linthicum et al. (1987) found that vegetational changes, in response to rainfall, can be identified by remote sensing from satellites and used to identify areas of virus activity and to forecast outbreaks of Rift Valley fever. Remote sensing may become very useful for monitoring a wide variety of other diseases that are associated with specific weather or vegetational changes. Increased precipitation in the form of snow may limit food availability, restrict animal movements, and cause animals to use unusual food sources, resulting in diseases such as rumen overload in deer and pronghorns feeding on grain (Wobeser and Runge 1975b) and aspergillosis in ducks feeding on mouldy ensilage (Adrian et al. 1978).

Wind may concentrate floating materials, such as toxic blue-green algae or oil, in areas used by wild animals and affect the distribution of disease in this way. Wind also may concentrate or disperse sick and dead animals, affecting the recovery of carcasses and, hence, estimates of mortality during disease investigations (Armstrong et al. 1978). Wind may influence the distribution of airborne infectious agents and contaminants. Some viruses, such as foot-and-mouth disease virus, may be carried in the form of aerosols for long distances (Gloster et al. 1982) and the arthropod vectors of other diseases have been transported for up to 300 km by wind (Pedgley 1983). Mapping of wind distribution in the form of wind rose patterns together with that of other disease events may be a very useful part of the investigation of many diseases. Lack of wind, as occurs when air-borne pollution is trapped near the ground by a temperature inversion, may also cause a problem. Lack of wind, resulting in snow-covered vegetation, has been cited as a factor in winter mortality of Dall sheep in the Yukon (Burles and Hoefs 1984).

Humidity is important for in vitro survival of many microbial agents and also for invertebrate vectors of disease, such as fleas (Cavanaugh and Marshall 1972) and the gastropod intermediate hosts of many parasites. Solar radiation also affects in vitro survival of microorganisms and may have direct effects on the energetics of animals (Lustick et al. 1979) and on the behavior of some parasitic disease agents (Hensley 1976).

5.1.3 Soil

Soils influence disease in many ways (Horvath and Reid 1984) but have received relatively little attention by wildlife disease investigators. The soil in an area reflects the parent bedrock and the long-term climate of the area and influences both the plant and animal species present on the area. Important characteristics of soil in relation to disease include its chemical, physical, and moisture composition, as well as factors such as its temperature and biotic components. The geochemistry of soil is associated with the distribution and occurrence of a number of diseases in humans (National Research Council 1974) and nutritional deficiencies in association with certain soil types are well documented in domestic animals. Knowledge of the role of minerals in diseases of wildlife is fragmentary but macro- and micronutrient deficiencies have been suspected or confirmed in wild species including deficiency of selenium (Hebert and Cowan 1971; Fleming et al. 1977; Shaw and Reynolds 1985), sodium (Botkin et al. 1973), copper (Flynn and Franzmann 1974; McDiarmid 1975), phosphorus (Bowyer 1983; Hanley and McKendrick 1985), and calcium (Phalen et al. 2005). Much of the work to date in wild species has depended upon extrapolation from domestic livestock. Base-line or normal concentrations of elements in the tissue of wild animals are largely unknown, as are the nutritional requirements of the animals and the effects of deficiencies. The role of trace minerals and other nutrients in resistance to infectious diseases may be particularly important. Studies of base-line values, such as that of Franzman et al. (1977) and of experimentally-induced deficiency (Brady et al. 1978), are needed. Poisoning may occur because of high levels of elements such as fluoride (Shupe et al. 1984) and cadmium (Klok et al. 2000) in soils or because of toxic plants associated with particular soil types (Fowler 1983). Acid precipitation may alter the availability of nutrients and toxic materials in soil. Acid precipitation has been associated with calcium deficiency in passerine birds in some areas (Drent and Woldendorp 1989) but not in other areas (Ramsay and Houston 1999). Klok et al. (2000) compared the risk of cadmium poisoning in badgers in areas with acidified and non-acidified soils.

The distribution of some infectious diseases appears to be related to soil/bedrock geochemistry; one well-documented example in wildlife is the association between the distribution of leptospirosis and Paleozoic bedrock in Ontario (Kingscote 1970). Soil may act as a reservoir or habitat for infectious agents and specific soil types are associated with particular agents. For example, the fungus *Blastomyces dermatitidis* has been associated with sandy, acid soils (Thiel et al. 1987) and *Yersinia* spp. are found in areas characterized by specific soil and vegetation types (Botzler 1979, 1987). Areas where the tick *Ixodes scapularis* (that transmits Lyme disease) is likely to become established can be predicted based on soil type and vegetative cover (Guerra et al. 2002).

The physical characteristics of the soil also may be important in some diseases. For example, lead shot persists within the reach of waterfowl longer

in ponds with a firm bottom than in ponds with a soft bottom because the shot sinks out of reach of the birds more rapidly in the latter situation (Bellrose 1959). Carey et al. (1980) found that the soil depth and moisture influenced both the rodent hosts and the tick vectors of Colorado tick fever.

When all of the possible interactions between soil and disease are considered, it is apparent that most disease investigators will require assistance from soil specialists to examine this factor in detail. Both general and detailed information on the soils in a region are usually available from government and university agricultural agencies and qualified specialists are available in these institutions.

5.1.4 Water

Water is intimately involved in so many aspects of disease that it is difficult to identify examples of disease in which water does not play some role. Consideration of water should be a part of every disease investigation. Surface water modifies climate, soil and vegetation, and often determines the distribution of both animal and human activities in an area. Water provides habitat for many infectious disease agents, and for their hosts or vectors. Even infectious agents that are not normally thought of as being aquatic survive longer in water than in the terrestrial environment. Animals congregate in dense aggregations about water sources, promoting transmission of infectious agents within a species and exchange of agents among species that are spatially separated at other times. Water is a fomes or carrier for many infectious diseases and may carry or concentrate harmful substances of both natural origin (e.g., mercury, fluoride) and from anthrogenic sources (e.g., oil, pesticides). Water also may transport and disperse infectious and non-infectious agents over great distances. The arthropod-borne viral diseases provide striking examples of the importance of even tiny and ephemeral bodies of water, such as that collected in the leaf axil of a plant or in discarded automobile tires, in the ecology of a disease. When evaluating the role of water in disease events, temporal changes in level, temperature, chemistry and biota must be considered. Almost all surface water available for wildlife has been modified by human activity. This aspect will be considered in some detail later in this chapter.

5.2 Characteristics of the biotic environment

5.2.1 Vegetation

Many diseases of wildlife have a strong association with a particular ecosystem that is characterized by certain types of vegetation. In diseases caused by consumption of a toxic plant, such as locoism in elk (Adcock and

Keiss 1969), the connection between plant and disease is direct and easy to understand. In other diseases, the association is less direct. For example, the fungal disease adiaspiromycosis in ground squirrels has a strong association with brushy habitat. Squirrels in brushy habitat have a much higher prevalence of infection than do squirrels in adjacent grassy areas. This is probably because the fungus forms part of the root-associated mycoflora of certain woody plants, so that Franklin's ground squirrels living under shrubs have a much greater exposure to the fungus than do Richardson's ground squirrels living under grass (Leighton and Wobeser 1978). In most diseases, the association is even less direct and it is often difficult to separate the influence of climate and soil from that of vegetation. This is not surprising, as the vegetation of an area is determined by the climate and soil, and the plants present modify both the soil and the microclimate on a site. Thus, when a pathogenic bacterium, such as *Yersinia enterocolitica*, is found in the soil of forested areas but not in the soil of grassed areas (Botzler 1979, 1987), it is unclear if there is a cause-effect relationship between the distribution of vegetation and the bacterium, or if both are affected by some other factor(s). Similarly, the density of *Ixodes scapularis* is influenced both by the forest type and the soil type (Guerra et al. 2002). As noted earlier, remote sensing by satellite will probably become increasingly useful in the future for mapping and predicting the occurrence of diseases as we identify associations between vegetation and specific diseases.

Parelaphostrongylus tenuis provides a good example of the complexity and difficulty in separating the effects of various factors. This nematode is distributed widely in eastern and central North America. It is generally limited to areas with deciduous or mixed deciduous/coniferous forest and does not occur in areas with predominantly pine forests or in grassland areas (Kocan et al. 1982). The distribution of the parasite appears to be related to the availability of suitable molluscan intermediate hosts (Lankester and Anderson 1968), although this may not be the only limiting factor in some areas (Kocan et al. 1982). Even in areas where apparently suitable mollusks occur in all habitat types there is a higher prevalence of infection among gastropods in deciduous and deciduous/mixed habitats than in other habitats (Kearney and Gilbert 1976; Upshall et al. 1986). White-tailed deer in these habitat types have a higher prevalence of infection than do deer in other areas (Thurston and Strout 1978). The mechanisms responsible for the difference in occurrence of the parasite in different habitats are unknown but probably relate to soil and surface moisture conditions and, hence, to the suitability for gastropods. General climate may be important in this regard, and Upshall et al. (1986) suggested that leaf litter in deciduous habitats may provide a moist refuge under which gastropods can estivate during hot, dry weather. Thus, plant-derived microenvironment is likely also important. The parasite generally is not found in areas with sandy soil (Kocan et al. 1982), perhaps due to good surface drainage. The prevalence of infection in deer in enzootic areas varies with the annual rainfall (Behrend and Witter 1968; Gilbert 1973),

which indicates an influence by both soil and weather. Selection and usage of different habitat types by deer, the final host, is undoubtedly also of importance in determining the distribution of the parasite. Ecologic separation based on the use of different habitat types may reduce the transmission of the parasite from deer to moose (Kearney and Gilbert 1976; Upshall et al. 1987) and from deer to elk (Raskevitz et al. 1991).

Even in simple systems, it usually is much easier to identify an association between a vegetation type and a disease than to discover the mechanism responsible for the association. For example, Getz (1970) found a striking difference in the prevalence of infection with botfly larvae (*Cuterebra augustirons*) in meadow voles in two adjacent areas of a marsh. The major difference between the areas was that one was mowed annually while the other had not been mowed for some years. The author felt that the difference in prevalence of the parasite was connected in some way to the leaf litter layer; however, the cause for the phenomenon was not identified, although several environmental variables were measured.

Despite difficulties in identifying the causative mechanisms, characterization of disease by defining associations with vegetation types or ecotypes is a useful technique for the disease investigator. This is particularly so for predicting the occurrence and/or spread of a disease into new areas, and for planning both investigation and management programs. Obviously, if an animal or a disease is associated with a particular vegetation type, sampling, monitoring and management procedures should be directed at that type of habitat. In the early stages of an investigation, it may be necessary to sample all habitat types in an area to determine the general distribution of animals and disease. Based on this information, further sampling can be done in a stratified manner, in the same way that population censuses are done. In this way, efforts can be directed at those areas in which there is the greatest probability of detecting the disease. For example, if one were interested in discovering if *P. tenuis* was present in a region, it would be sensible to begin by surveying areas with clay soil and deciduous forest, rather than to begin looking in grassland areas with sandy soil. Identification of links between vegetation and disease may be important in planning disease management, e.g., the rate at which rabies spreads geographically among raccoons is influenced by forest cover. Smith et al. (2005) have suggested that rabies control could be enhanced by focusing vaccine bait distribution along rivers in lightly forested areas.

Most disease investigators are not trained botanists but the ability to recognize vegetation types and common plant species should be part of the woodcraft of every investigator engaged in field research. Various ecosystems have been described in detail (e.g., Cowardin et al. 1979) and techniques for evaluating habitat that are suitable for disease studies are available in Flood et al. (1977) and Cooperrider et al. (1986). Whenever possible, habitat features and types should be described using standardized nomenclature. The professional assistance of botanists, foresters, range managers, agriculturalists, and land-use ecologists should be sought for detailed studies.

5.2.2 Animals

It is impossible to predict in advance what aspects of the faunal community of an area may be involved in a disease. Hence, it is difficult to develop a set of general procedures for investigating this aspect of the environment. During most investigations, attention is focused first on the vertebrate species that are obviously involved and usually it is only later that the role of other species is recognized. However, some of these peripheral animals may be very important in the ecology of the disease. For instance, during an outbreak of avian botulism, only a few species of birds may have clinical disease. The birds are poisoned by ingesting toxin produced by the bacterium *Clostridium botulinum* when it grew within the anaerobic environment of decaying organic matter. Animal carcasses are a particularly good substrate for toxin production and an outbreak of botulism might be triggered by any event that provides sufficient carcass substrate in a marsh. Spores of *C. botulinum* are common in the soil of marshes (Wobeser et al. 1987) and animals living in a marsh are likely to have spores in their digestive tract. Thus, events as diverse as mortality of aquatic invertebrates or fish because of unfavorable water conditions, death of terrestrial invertebrates because of sudden flooding, death of birds because of collision with overhead wires above the marsh, a hail storm, or hunting, or even the presence of a cow carcass in the marsh (Hunter and Clark 1971) might provide suitable substrate for toxin production. As an example, nestling Franklin's gulls that died from other causes provided a source of toxic maggots that coincided with the first cases of botulism in ducks in each year of a 3-year study (Soos and Wobeser 2006). The amount of substrate available for toxin production is modified by the activity of scavengers that remove carcasses before toxin is formed. Toxin is transferred from vertebrate carcasses to birds through the intermediary of maggots (sarcophagus fly larvae) that contain toxin and are readily consumed by birds. Thus, the ecology of botulism in a specific marsh may be influenced by the many species that might act as carcass substrate, by the number and activity of scavenging species, by the number and activity of blowflies and other sarcophagus invertebrates, and by the type and number of birds present to serve as victims.

An initial step in the investigation of a disease is to catalogue the species of vertebrates present on the area, and to categorize the animals into two groups: those affected by the disease and those that seem unaffected. This may be relatively simple to do during an outbreak of an acute disease in which affected animals have obvious clinical signs, but it may be very difficult in chronic diseases, conditions characterized by mild or subtle effects, or conditions in which subclinical disease is common. When the species have been categorized in this way, one then can begin to search for **common features within** each of the groups and **differences between** the affected and the unaffected groups. Two obvious features that should always be examined are the **taxonomic relatedness** and the **ecologic niche** of the animals within each of

the groups. Closely related species tend to be susceptible to many of the same diseases, and animals occupying a similar ecologic niche often have similar 'occupational' exposure to certain disease factors. For example, assume that during the investigation of an unknown disease we find that coyotes, red foxes, and wolves are affected, while black bears in the area are not. A common feature of the affected group is that they are all members of the family Canidae, whereas the unaffected bears are of a different taxonomic group. A disease such as canine distemper that affects canids but has not been reported in bears might produce this type of pattern. Conversely, osprey, river otter, and great blue herons, although taxonomically diverse, all eat fish, and might be affected by a toxin such as mercury that accumulates in fish, while closely related species that do not eat fish would be unaffected. Ecological similarity among hosts may be more important than taxonomic relatedness in determining susceptibility to macroparasites (Poulin 2005).

The affected and unaffected groups can be compared with respect to an infinite number of other variables. One should be looking for common features within groups and differences between groups. Exceptions to a general rule also may provide valuable clues for understanding a disease. For instance, if all species of raptorial birds in an area, except one, have residues of a particular pesticide in their tissues, examination of how that species differs from the others in features such as diet, feeding habits, preferred habitat, and migration route and timing might provide an important lead to the source of the chemical. This simple technique of classifying animals into affected and unaffected groups, and then looking at the features of each group is particularly valuable during the early stages of an investigation when one is looking for any clue to the nature of the disease involved. During the investigation of a die-off of birds at a local game-park, we noted that both ducks and gulls were dying. Ducks of the same species were present in both the affected and unaffected groups, and the only obvious difference between the groups was the ability to fly from the pond. Only free-flying birds were affected, while pinioned, flightless waterfowl resident on the pond were unaffected. This suggested that the affected birds were being exposed to a disease agent somewhere other than on the pond. We subsequently found that the free-flying birds were dying of strychnine poisoning as a result of consuming grain bait used for rodent control in nearby fields.

In the case of infectious diseases that infect a number of host species, it is important to identify the role that each of the affected species plays in the ecology of the disease. Not all species that are infected with the agent may be involved in transmission or perpetuation of the disease. Hosts usually can be assigned to one of three types: **maintenance hosts** are those that can perpetuate the disease indefinitely without introductions from an outside source; **spillover hosts** may be involved in transmission to some degree but can not perpetuate the disease indefinitely without introductions from another source; **dead-end hosts** are those that may become infected from another source but do not transmit the disease further. Caley and Hone (2005)

suggested that the status of a host animal can be determined by estimating the basic reproductive rate of the disease (R_0) in that species. R_0 will be discussed later, but it is defined as the average number of secondary infections produced when one infectious individual is introduced into a totally susceptible population. In maintenance hosts $R_0 \geq 1$; in spillover hosts $0 < R_0 < 1$, and in dead-end hosts $R_0 = 0$. Determining the actual status of the species involved may be difficult and a host may serve as a maintenance host in one circumstance and as a spillover host in another situation (Caley and Hone 2005).

The principle of comparing affected and unaffected groups or individuals should be applied within the affected species. Members of the same species are an important component of the environment, and intraspecific behavior, competition and aggregation may be major influences on disease. Differences in behavior, as well as factors such as population number, density, distribution and composition, as well as the prevalence, incidence, mortality and survival rates of various subgroups within the affected population are all parameters to be examined. As an example, there was a significant difference in organochlorine pesticide residues in glaucous gulls nesting in two colonies about 1–2 km apart on an island (Bustnes et al. 2000). This was found to result from a difference in feeding ecology and trophic level; birds with the higher level of residues fed extensively on eggs from an adjacent guillemot colony, while gulls in the other colony fed more extensively on fish.

The nature of the specific disease will usually determine the role that other animal species play in its ecology. In general, when dealing with infectious diseases, one is concerned with species that may act as causative agents of disease, hosts of different types for the causative agent, and/or transmitters of the causative agent. In some situations, a single species of animal may fulfill more than one of these roles. Duncan et al. (1978) studied the role of the tick *Ixodes ricinus* in disease among red grouse in Scotland and found an inverse relationship between the number of ticks on grouse chicks and the breeding success and population density of grouse at different sites. They concluded that ticks affected grouse in at least two different ways. Chicks <14 days old died as a direct result of injuries and blindness caused by the ticks, so that the ticks were a causative agent of disease for this age of bird. The ticks also were both the alternate host and the transmitter (vector) of louping ill virus that may have been responsible for a high mortality rate among adult hens.

When considering the possible involvement of other species in a disease, it is important to remember that: (i) alternate hosts of an infectious disease may not show any ill-effects of the infection, and (ii) the disease produced in these species may be considerably different than that in the animal under study. For example, a herpesvirus that causes fatal malignant catarrhal fever in cattle and some cervids causes no detectable disease in its normal host, the wildebeest. Such silent infections in alternate hosts are particularly difficult to detect.

When dealing with non-infectious diseases, particularly toxicity problems, one should consider species that may play a role as a causative factor, as

accumulators or concentrators of toxic material, and/or as transmitters of disease. Venomous or toxic species may cause disease directly. The bioaccumulation and amplification of toxins within food-chains, as evident in the example of the glaucous gulls referred to above, is such a common phenomenon that this should be considered in any investigation of a toxicity problem. Investigation of many toxicity problems may involve starting at the species recognized to be affected and then working down the food-chain to identify species that may act as accumulators, and up the food-chain to identify potential candidates for secondary or tertiary poisoning. As noted in the discussion of botulism, animals also may act as transmitters of toxin. Fly maggots are not affected by the botulinum toxin but as few as three or four maggots may contain sufficient toxin to kill a duck.

Animal species also may influence disease through more general mechanisms, such as the direct or stressor effects of predation, competition for resources, or through modification of the shared environment. The latter effect may be particularly dramatic and diverse. For example, burrows of one species may provide the nidus for survival of pathogens that affect other animals; soil enriched with feces of birds or bats provides the microenvironment for the opportunistic fungus *Histoplasma capsulatum*; and vole urine has been found to modify the pH of soil, allowing *Leptospira* spp. to persist in otherwise inhospitable areas (Horvath and Reid 1984).

When dealing with an unknown disease, one often has to work initially by extrapolation and analogy from similar diseases and by using knowledge of the biology of the animals involved. For instance, if confronted with a new pathogenic fluke (trematode) in ducks, it would be reasonable to expect that gastropods would be involved in the disease because gastropods are involved in other diseases caused by flukes. Similarly, if one was studying a new species of the genus *Sarcocystis* in rabbits, it would be logical to expect that a predator of rabbits would be the alternate host of the parasite because all members of the genus *Sarcocystis* have an obligate predator–prey life cycle. If confronted with insecticide residues in insectivorous birds, one would check the use of insecticides to control insects of the type eaten by these birds, and might also examine bird-eating raptorial birds for possible secondary intoxication.

5.3 Human effects on disease

Although the thought is unpleasant, it is important to realize that totally natural areas no longer exist and that all wild animals live in environments that are modified by humans. The extent of this modification varies from the ubiquitous occurrence of certain persistent contaminants, even in otherwise pristine arctic and antarctic regions, to the totally artificial environment used by many species of urban wildlife. Humans influence the occurrence of most diseases of wildlife in some way but the impact is often indirect through

effects on other elements of the environment. An important consideration in assessing the significance of human factors is the rapidity with which anthropogenic changes occur relative to an evolutionary time scale. Wild animals can adapt to altered environments, e.g., moths have adapted through 'industrial melanism' to the sooty environments associated with industrialization (Kettlewell 1971) and wild rabbits in various areas have developed a degree of genetic resistance to artificially introduced myxomatosis (Ross 1982); however, there is little information available to suggest that wild species have been able to accommodate to polychlorinated biphenyls, or to other major environmental changes, such as widespread occurrence of plastic garbage in the oceans of the world.

The most direct effect of humans on disease in wildlife is the occurrence of intoxication as a result of the release of contaminants into the environment. In the great majority of instances, intoxication is inadvertent, although planned or malicious poisoning of wildlife also occurs. The basic methods for investigation of toxicologic disease are not different from those used for other types of disease. However, the study of environmental toxicology is complicated by the great variety of potential toxins in the environment, the simultaneous occurrence of residues of many compounds in the tissues of wild animals, lack of knowledge of the effect of most of the substances on particular wild species, and the great variability in the response of different species to any specific compound. In general, it is easier to establish the relationship between a highly toxic compound and the disease it produces than it is to link compounds of low direct toxicity to the subtle or sub-lethal disease that they may produce. It is usually more difficult to relate disease to compounds that are widely dispersed than to compounds that originate from a single or point source. Pollutants acquired at distant locations, e.g., during migration, present special difficulties for investigators (Babcock and Flickinger 1977; White et al. 1983; Anderson et al. 1984).

Very little is known about the effect of combinations of various contaminants on wildlife, although many wild animals are exposed to a plethora of substances and poisoned animals may carry a cocktail of substances in their tissues (e.g., Dieter and Ludke 1975, 1978). Contaminants at sublethal levels may reduce resistance of wild animals to infectious diseases (Friend and Trainer 1972a, 1972b; Zeakes et al. 1981; Trust et al. 1990) and act synergistically with other environmental factors such as cold (Fleming et al. 1985), but these effects are even more difficult to detect and prove than is direct toxicity.

Humans also may influence disease conditions in wild animals directly through the introduction or translocation of disease agents, vectors, and wild or domestic animals. In a few instances the movement of disease agents has been intentional, as in the introduction of myxomatosis and rabbit hemorrhagic disease into Australia to reduce rabbit populations and the less publicized introduction of feline parvovirus to control feral cats on Marion Island (Van Rensburg et al. 1987). In most cases, introductions have been inadvertent through ignorance or indifference. Entirely too many examples of this type of carelessness exist and unfortunately continue to occur. Translocation

and introduction of diseases is an important and potentially controllable factor that will be discussed in detail in Chap. 15.

Most effects of human activity on disease occur as a result of modifications to some other aspect of the environment. The effects of modifications to water will be discussed in detail but many of the same principles are applicable to changes in other environmental features. Surface waters are continually manipulated in amount, distribution and quality. Stanley and Alpers (1975) provide dramatic examples of the effect of man-made lakes on infectious diseases (viral, bacterial, and parasitic) of humans. The effects of water manipulations on diseases of wildlife are likely to be no less dramatic, but have been poorly documented to date. Both artificial increases and decreases in water in an area may affect disease. Ebedes (1977) described the effects of water management on the occurrence of anthrax in a South African park. Under natural conditions, large mammals in the area migrated extensively during the dry season and the population density was low. To enhance tourism, artificial waterholes were constructed near roads, resulting in many animals occupying these areas over an extended period. This caused severe habitat destruction in the area and, following introduction of anthrax by cattle, the water holes became enzootic nidi for the disease. This resulted in large-scale mortality among the concentrated wild animals. A less dramatic example of the effect of water manipulation on a disease is the creation of breeding sites for *Culicoides variipennis* in runoff water from certain agricultural activities, which resulted in difficulty in controlling bluetongue virus infections carried by these insects (Jones et al. 1981).

The most dramatic impact of water manipulation on disease will likely occur among water birds. Here, the problem is usually a combination of too little water and water of poor quality. Another problem may be the reduction of resources in water required by the birds, e.g., over-fishing of horseshoe crabs in Delaware Bay has had dramatic population effects on migrating red knots dependent on this resource to refuel during migration to the arctic (Baker et al. 2004a). The continual loss of natural wetlands throughout the world from drainage and human encroachment has resulted in compression of bird populations onto residual refuge areas. This effect is particularly severe in wintering areas in North America, but also occurs on staging areas used during migration and on the breeding grounds. For instance, about 90% of the entire mid-continent population of sandhill cranes roosts during winter on nine small lakes in Texas (Iverson et al. 1985). Inadequate flow in the Platte River in Nebraska, as a result of dams, together with resulting encroachment by vegetation, has resulted in a situation in which 400,000 of these cranes congregate at densities exceeding 625 birds/100 m of channel for several weeks during spring migration (Krapu and Pearson 1982). Continued low flow in the river might result in a major shift of cranes to the nearby Rainwater Basin area, an enzootic focus of avian cholera that is already used by 5–9 million ducks and several hundred thousand geese (Windingstad et al. 1983). Maintenance of animals at such high densities for extended periods of time enhances the transmission of infectious agents, promotes exchange of diseases among populations and species, and the stress

of high density may also lower the resistance of individuals to infection (Ould and Welch 1980). Any outbreak of disease that occurs under such a situation may endanger a significant proportion of the total population of a species, simply because all the eggs are in one basket.

Water is a finite commodity and the water available for wildlife often "*represents a reuse of water from municipal, agricultural or industrial sources*" (Friend 1981). Scarcity of natural wetland habitat has resulted in suggestions that reused water in sewage lagoons (Maxson 1981; Piest and Sowls 1985) and from industrial processes such as oil shale projects (Snyder and Snyder 1984) should be developed as replacement habitat for wild animals. Dramatic problems such as selenium poisoning of birds as a result of agricultural runoff water in the Kesterson area of California (Ohlendorf et al. 1988; Zahm 1986) probably represent an extreme example of the type of situation that may occur when animals use wastewater. Other less publicized examples include mortality of ducks from detergents (Choules et al. 1978), DDT contamination of a refuge by industrial wastewater (O'Shea et al. 1980), botulism in gulls in association with rubbish dumps (Lloyd et al. 1975), infection by the nematode *Eustrongylides ignotus* in wading birds using wetlands enriched by nutrient pollution (Spalding et al. 1993), and contamination with bacterial pathogens including *Salmonella* spp. (Steiniger 1962), *Yersinia enterocolitica* (Kapperud and Olsvik 1982) and *Clostridium* spp. (Ankerberg 1984) associated with sewage. These examples are intended to indicate that a disease investigator should **always** be concerned about the quantity, source, and quality of water available for the wild species under study. References such as standard methods for the examination of water and wastewater (American Public Health Association 2005) should be as familiar to the investigator as are more orthodox references sources on infectious diseases and zoology.

While humans may not be able to markedly modify the weather in an area, microclimate is often altered extensively and this may have a profound effect on disease. For example, irrigation, in addition to altering the vegetation and soil in an area, also increases the local humidity and this may allow the gastropod intermediate hosts of a number of parasites, such as the liver fluke *Fascioloides magna*, to expand their geographic range into new areas. Alterations in vegetation through agriculture or forestry have obvious effects on the distribution of animals. Soils are altered through agricultural practices, such as cultivation and fertilization, and also may be altered by atmospheric fallout from distant industrial activities. Atmospheric sulphur from fossil-fuel combustion, falling as acid rain, may cause a decrease in the level of selenium in vegetation (Frost and Ingvoldstad 1975) raising concern about nutritional deficiency in wild animals (Shaw and Reynolds 1985) and acid rain has been associated with abnormally high concentrations of cadmium in the tissues of wildlife (Froslie et al. 1986, Klok et al. 2000) and with calcium deficiency in birds (Drent and Woldendorp 1989).

Management practices such as artificial feeding, which concentrate mammals, cause the same general problems alluded to in the concentration of

waterfowl. The occurrence of diseases such as necrobacillosis (Rosen 1981), brucellosis (Thorne et al. 1982), and tuberculosis (Miller et al. 2003) among cervids concentrated by artificial feeding illustrate the risks inherent in artificial concentration. Even unintentional feeding, such as at garbage disposal sites, may result in disease. Conlogue et al. (1979) described nutritional bone disease in Arctic fox pups scavenging at a seal carcass dump, and botulism occurs among gulls feeding at rubbish dumps (Lloyd et al. 1975). Enhanced contact between wild and domestic animals may also promote the exchange of a variety of diseases in both directions.

This section has contained examples of ways in which human activities may influence disease in wildlife. It should be obvious that the disease investigator must be very cognizant of human activities that may affect the disease or species under study. In most areas, agriculture is the single most significant activity that affects wildlife. Unfortunately, wildlife and agricultural interests often take adversarial positions but such animosity may be a luxury the disease investigator cannot afford. Knowledge and understanding of agricultural practice in the area will assist the investigator in many ways. Agricultural crops form both the habitat and the food base for many wild species, so that recognition of these must be a part of woodcraft of the investigator, in the same way that he or she should know native plants. Many toxicity problems are related to agricultural chemicals, so that an investigator should be aware of which chemicals are used at what time of the year and on which crops. The control of certain diseases also may rest in inducing changes in agricultural practice. For instance, Windingstad et al. (1989) found that a problem with mycotoxicosis in sandhill cranes was reduced by encouraging farmers to bury mouldy peanuts by plowing. Harvesting grain crops so that the remaining stubble is too tall to be used by geese has been used to segregate field-feeding cranes from geese, to reduce the probability of transmission of avian cholera between the two groups (Windingstad 1992, personal communication). Finally, many wild animals live on private agricultural land. Farmers and ranchers are active on this land and can be valuable allies in detecting disease problems and in identifying problem situations. The ability to discuss agricultural concerns intelligently is a valuable asset in establishing credibility and in gaining both their assistance and access to the land. Information on many aspects of the agricultural environment, such as soils, crops, and cropping practices, is available from district agriculturalists and agricultural extension workers and disease investigators should establish a working relationship with these people.

5.4 Summary

- Diseases are restricted in distribution by features of the host population, the causative agent or factor, and the external environment. These features are often so intertwined that it is difficult to assess individual factors.

- Definition of the environmental factors that influence a disease is a vital part of any investigation. Because of the complexity of the interactions, involvement of specialists in many disciplines usually is needed.
- Characterization of environmental factors usually begins with broad features, such as weather, and proceeds to more local factors, such as the microclimate within an animal's burrow.
- Important abiotic features that should be assessed include topography, climate, weather, soil, and water.
- Water is involved in the ecology of almost every disease; through effects on climate, vegetation, and animal and human distribution; by providing habitat for disease agents and vectors; and by carrying and concentrating infectious agents and toxic substances. Every disease study should include a consideration of its relation to water.
- Biotic factors that should be assessed include vegetation, animals, and human activities.
- Animals may be involved as causative agents, reservoirs, vectors or transmitters of disease and may also influence disease through predation, scavenging, competition and habitat modification.
- An early step in assessing the role of animals is to divide species into those that are involved and those that seem not to be involved, and to compare taxonomic and ecologic characteristics of the two groups in order to identify features associated with the disease.
- In infectious diseases, it is important to clarify the role of the various species that may be infected as maintenance, spillover or dead-end hosts.
- Humans influence disease by introduction of toxins or disease agents into the environment, by moving disease agents, vectors and animals, and by modifying other aspects of the environment.
- Because many human impacts are related to agriculture, the investigator should have a working knowledge of agricultural practice in the area and work cooperatively with agricultural specialists.
- The disease investigator should expect to find unexpected and unprecedented interactions among environmental factors and disease.

6 Formulating and testing hypotheses

"*Construction of a hypothesis implies a belief that there exists a degree of order or regularity that can be identified and measured despite fluctuations in response*"

(Skalski and Robson 1992)

6.1 Hypotheses

The term hypothesis has been mentioned several times in the preceding chapters. Hypothesis has many meanings, ranging from any speculative thought to "*concrete, specific conjectures on the process that lead to an outcome*" (Guthery et al. 2004). The definition I will use is that a hypothesis is a proposition set forth as an explanation for the occurrence of a specified phenomenon. The basis of scientific investigation is the collection of information that is used either to formulate or to test hypotheses. One assesses the important variables and tries to build a model or hypothesis that explains the observed phenomenon. In general, a hypothesis is formulated by rephrasing the objective of a study as a statement, e.g., if the objective of an investigation is to determine if a pesticide is safe, the resulting hypotheses might be that 'the pesticide is not safe' or that 'the pesticide is safe'. A hypothesis is a statistical hypothesis if it is stated in terms related to the distribution of populations. The general hypothesis above might be refined to: 'this pesticide, when used as directed, has no effect on the average number of robins in an area', which is a testable hypothesis. The hypothesis to be tested is called the **null hypothesis** (H_0). The **alternative hypothesis** (H_1) for the above example would be 'this pesticide, when used as directed, has an effect on the average number of robins in an area'. In testing a hypothesis, H_0 is considered to be true, unless the sample data indicate otherwise, (i.e., that the pesticide is innocent, unless proven guilty). Testing cannot prove H_0 to be true but the results can cause it to be rejected. Failing to reject the hypothesis does not mean that it is true. In accepting or rejecting H_0, two types of error may be made. If H_0 is rejected when, in fact, it is true a **type 1** error has been committed. If H_0 is not true and the test fails to reject it, a **type 2** error has been made.

The decision to accept or reject H_0 is made based on some estimated risk of being wrong in that decision, and usually the probability of making a

type 1 error (rejecting a true hypothesis) is of greatest concern. The probability of this error is called the level of significance of the test and the acceptable level of significance should be established prior to, rather than after, testing. The level of significance chosen in any situation is a subjective decision. In most areas of science, this is commonly set at 0.05, i.e., one accepts a 1 in 20 chance of being in error. The choice of a less rigorous test invites criticism and, in many instances, more rigor may be appropriate. However, in many situations dealing with wild populations, the investigator should ask himself or herself, quietly, if the methods available for counting animals and measuring other variables are really reliable enough to justify such confidence.

The choice of which of two alternative hypotheses to use as H_0 and which as H_1 is an important decision because, in statistical tests, H_0 is not rejected unless the evidence against it is overwhelming. In making the choice, one must consider which type of error (type 1 or type 2) is more critical in a biological or real world sense. If you were asked to test the safety of a pesticide, with licensure for widespread use depending on your results, the errors that might be made would (i) result in use of an unsafe pesticide that would risk animal and human health, or (ii) not allow use of a safe chemical resulting in higher costs of production for a crop. Most people would consider it to be far more costly to allow the use of an unsafe chemical than to disallow the use of a safe one. In this case, the appropriate decision should be that H_0 = the chemical is unsafe and H_1 = the chemical is safe, because, in this way, there is a smaller possibility of erring by allowing use of an unsafe chemical.

In other situations, where the risks are less well defined, the hypothesis that there is no effect is usually taken as H_0. For instance, if we were evaluating the efficacy of a new drug for potential use in the control of lungworms in bighorn sheep, we would likely choose that H_0 = the drug has no effect on the number of lungworms. This assumes that there is no effect and places the burden of proof on the pharmacologist (and the worms) to demonstrate an effect by disproving the hypothesis.

Hypotheses are tested by comparing them to observed data. When a hypothesis fails to meet or explain the data, one first checks the data, and then one tries to improve the hypothesis. This process is a continuous one of refining and retesting. In some instances, several competing hypotheses may be proposed and examined to see which one best explains some phenomenon. For instance, Caley and Hone (2002) developed a set of hypotheses or models that might explain how tuberculosis is transmitted to wild ferrets in New Zealand. They fitted these models to age-specific prevalence data collected in the field as a test of the competing hypotheses to determine which model best approximated the field situation. They found that consumption of tuberculous carrion or prey was the most strongly supported model for transmission to ferrets.

While I have presented the traditional approach of using statistical hypothesis testing, the reader should be aware that this approach has limitations and has been criticized for use in wildlife management (Johnson 1999;

Anderson et al. 2000; Guthery et al. 2001). Johnson (1999) raised serious concerns about the usefulness of statistical tests of hypothesis for ecological studies, and the need to be clear about the difference between statistical and biological significance. Alternative approaches, termed "*hypothesis-free science*" by Guthery et al. (2004), include purely descriptive studies, measures of magnitude of effect, and information-theoretic methods that provide strength information on multiple working hypotheses (models), all of which are plausible. Anderson and Burnham (2002) suggested that the need for modeling expertise in the latter of these is "*an excellent reason to seek the help of a statistician*".

The remainder of this chapter will be devoted to methods for collecting information about disease in populations, i.e., epizootiologic data, and very little will be said about collection of clinical information from individual animals.

6.2 Collecting information

Table 6.1 presents a schematic classification of the various methods used for investigating disease conditions. It should be recognized at the outset that there is considerable overlap among the various types and that individual investigations may involve elements of several types. However, each technique has inherent strengths and weaknesses that suit it for particular problems.

The most basic distinction is between **observational** and **experimental** studies. Observational studies are those in which information is collected about naturally occurring events and in which the investigator does not play an active part in what happens. In contrast, experimental studies measure the effect of manipulations caused by the investigator. To illustrate the difference, consider methods that might be used to study pneumonia in wild sheep. One method might consist of identifying and cataloguing the nasal microflora in bighorn sheep before, during and after a spontaneous outbreak of pneumonia. This is an observational study because the investigator is trying to study events as they occur, without manipulation. A second method might be to study the nasal

Table 6.1 Relationship among various forms of investigative methods that may be used in the study of disease

Investigation		
Experimental	Observational	
	Descriptive	**Analytical**
– laboratory experiment		– cross-sectional study
– field trial		– case-control study
– community trial		– cohort study

microflora before and after the sheep were treated with an antibiotic. This is an experimental study in which the object is to determine the effects of a manipulation. Both studies might be valuable in understanding pneumonia in sheep, and a combination of observational and experimental methods may provide the best information about a disease. As an example, Caley et al. (2001b) used both methods in a study of the relationship between the occurrence of tuberculosis in ferrets and the abundance of brushtail possums. In the observational portion of the study, the prevalence of tuberculosis in ferrets was found to be significantly related to the abundance of possums at a number of sites. When the abundance of possums was experimentally reduced, there was an 80% reduction in the odds of tuberculosis in ferrets in the years immediately after possum depopulation. The conclusion was that the transmission from possum to ferret accounted for most of the tuberculosis in ferrets.

Because scientists are not invisible observers, a problem in all observational studies is the need to minimize the unintentional manipulation that may occur during the investigation because of the presence of the investigator and any handling that may be required to mark animals. This was alluded to earlier in Chaps. 2 and 4, and will be mentioned periodically elsewhere. Whenever possible, the effects of manipulations on factors such as behavior and survival should be measured as part of the study and not assumed to have no effect. Examples of studies that measured the effect of some procedure involved in marking or handling animals are given in Table 6.2.

Table 6.2 Examples of studies that have measured the effect of procedures used for sampling, handling, or marking on wild animals

Species	Handling or marking procedure	Effect
Mallard	Radio-transmitter	Negative effect on reproduction and survival[1]
Wild turkey	Radio-transmitter	Negative effect on wing growth[2]
Moose	Radio-transmitter	No measurable on survival[3]
Grey partridge	Radio-transmitter	Adverse effect on survival, reproduction and body mass in some years[4]
White-winged dove	Radio-transmitter	No effect on blood parameters[5]
Big brown bat	Anesthesia, blood sampling	No measurable effect on survival[6]

[1] Paquette et al. (1997)
[2] Hubbard et al. (1998)
[3] Swenson et al. (1999)
[4] Bro et al. (1999)
[5] Small et al. (2005)
[6] Wimsatt et al. (2005)

Most of the emphasis in the biological sciences and, particularly in postgraduate training, is on experimental methods, so that I will assume that most readers are well acquainted with these techniques. Consequently, the emphasis here will be on observational methods, but many of the general features of sampling, data collection, and analysis apply to both types and individual studies often involve a mixture of observational and experimental elements.

6.2.1 Experimental methods

Before discussing observational methods, a few comments should be made about the various experimental methods. In Table 6.1, three such methods are indicated. In all of these, the investigator alters or manipulates one variable and then measures the resulting change in some other variable. The three methods differ in the way that subjects are chosen for inclusion in the trial, in the degree of control that the investigator has over other variables, and in the method that is used to assess change in the other variables.

It is easiest to explain these differences through the use of an example. Assume that we are interested in determining the efficacy of a vaccine for preventing disease caused by agent X. We could test this by any of the three experimental methods. In both laboratory and field experiments (the latter are usually referred to as clinical trials in human medicine), the experimenter controls the allocation of individuals to the principal and control groups. So, for a study using either of these methods, we might select 100 suitable animals and assign them randomly into two equal-sized groups. The 50 animals in the principal group would be vaccinated while the 50 animals in the control group would not be immunized. To this point the methods are the same but they differ in the technique that is used to test or **challenge** the vaccine. We want to test the efficacy of the immunization. Using the **laboratory experiment** method, each of the 100 animals would be administered a standard challenge dose of agent X and we would determine the effectiveness of the vaccine by comparing the results of this experimental infection in the principal and control groups. The investigator in a laboratory experiment controls all aspects of the challenge (dose, route, timing, etc.). In contrast, if we were to use the **field trial** method we would mark and release all 100 animals back into the wild after having immunized the 50 animals in the principal group. Challenge would occur through natural exposure to agent X and we would have no control over which, or how many, of the animals were exposed. Nor could we control the dose, route or timing of exposure. We would determine the effectiveness of the immunization by measuring and comparing parameters such as the survival time and rate of animals in the two groups, using some of the techniques discussed in Chap. 4 (this assumes that we would be able to find the animals again after release!). In both laboratory and field experiments, the effect of the manipulation is measured by the response in

individual animals. A study of the survival of raccoons immunized against rabies and released into the wild (Brown et al. 1990) is an example of a field trial.

If we were to use a **community trial**, the vaccine would be made available to animals in the area or community, perhaps in the form of an oral bait. We, as investigators, have no control over which or how many animals will consume the bait or become immunized. Challenge of the animals occurs through natural exposure to agent X, as in the field trial. Assessment of the results is done by measuring some indicator of disease occurrence in the **population**, such as the incidence rate, following application of the vaccine to the community. Comparisons might be made to the incidence rate in the population prior to attempted immunization or to the incidence in areas or communities where vaccine was not supplied. Brochier et al. (1988) used this method to study the efficacy of oral rabies vaccination of foxes in Belgium. In an experiment referred to earlier, Caley et al. (2001) reduced possum populations and measured the effect by monitoring the prevalence of tuberculosis in ferrets, but the investigators had no control over which ferrets were exposed to the disease. The important differences that distinguish community trials from other experimental methods are that: (i) the investigator does not choose or allocate which individuals will participate in the trial and, (ii) the effect of the manipulation is measured in the population rather than in the individual.

Laboratory experiments have been used extensively in the study of disease in wild animals but neither field trials nor community trials have been used widely. A few specific examples will be discussed later in this chapter because they contain elements of both experimental and observational techniques. Extensive guidelines for the conduct of clinical trials are available in epidemiology texts such as Martin et al. (1987) and Thrusfield (2005).

Intervention trials, involving experimental treatment of one segment of a free-ranging population to remove or reduce the effect of a disease agent are a very promising form of field trial for collecting information on the impact of disease on individuals and on a population. Good examples are studies in which selected groups of free-ranging red grouse (Hudson 1986; Hudson et al. 1992) and snowshoe hares (Murray et al. 1997) were treated with an anthelmintic to control parasites. Reproduction in the treated grouse was shown to be superior to that of untreated groups, and treated hares survived at a higher rate that untreated hares.

6.2.2 Observational methods

Observational studies can be **descriptive** or **analytical.** Descriptive studies, as the name implies, involve the description of disease-related events in a population, as well as the identification of those characteristics that define the particular disease. Descriptive studies usually dominate the early stages of an investigation and provide the preliminary data upon which hypotheses may be formulated. For example, during an outbreak or outbreaks of a disease, the

species, sex and age composition of the affected individuals might be defined, and the pathologic features and presence of potential causative agents could be described. For instance, the first step in defining the nature and cause of avian vacuolar myelinopathy was a detailed description of the pathology in affected birds (Thomas et al. 1998) that allowed identification of birds with this disease. If suitable population parameters are known, various rates (morbidity, mortality, prevalence) may be calculated. Associations between factors may be observed or described but the strength of these associations is not tested in purely descriptive studies.

In reviewing literature available on disease in wild animals, it is apparent that the overwhelming bulk of the information is descriptive in nature, reflecting the comparative youthfulness of the science. Descriptive studies are necessary (Herman 2002) and provide the basis for formulating hypotheses about disease that can then be tested. Thus, the stage is set for more widespread use of analytical methods in the study of many diseases of wild animals.

Analytical studies are based upon **comparison** between or among groups that differ in one or more variables. These investigations attempt to **explain** the relationship between disease-related variables and to **measure** the strength of observed associations. Three sub-types of analytic investigation are recognized, based primarily on the manner in which groups are chosen for comparison.

6.2.2.1 Prevalence surveys

The first of these is the **prevalence survey** or **cross-sectional** study, in which data are collected from a broad sample or cross-section of individuals from the population at large. This sample is then sub-divided into two or more sub-groups, based on the presence or absence of some variable. The most common variable used, in our context, is the evidence of disease. Prevalence surveys are concerned with existing disease, i.e., disease present at the time of the survey. Animals that have the disease are designated as **cases** and individuals within the sample that are free of the disease at the time of sampling are included in the **non-cases** or **control** group. The various categories, such as diseased, must be defined in unequivocal terms prior to data collection, so that individuals can be placed into the proper category.

As an example, consider a situation related to lead poisoning in ducks. Descriptive studies have noted the common occurrence of anemia among lead-poisoned ducks and it is thought that this aspect of the disease (anemia) and the risk factor (lead) are associated. A working hypothesis might be that lead causes anemia and a null hypothesis might be that: ‘the number of circulating red blood cells in ducks with and without a toxic concentration of lead in their blood is not different’. This implies that lead, at levels causing other signs of intoxication, has no effect on the number of circulating red blood cells in ducks. One approach would be to examine a sample or **cross section** of

ducks from an area where spontaneous lead-poisoning occurs. For this study, anemia, which is the dependent variable, is defined by the number of red blood cells in circulation, measured by determining the packed cell volume (PCV) of a centrifuged blood sample. The independent variable is exposure to lead, measured by analyzing the concentration of lead in whole blood. We must establish unequivocal criteria for each category in advance of the study. On the basis of published literature we might decide that the diagnostic level for lead poisoning will be a concentration 10 ppm of lead in blood and that any duck with a PCV ≤.320 L/L will be considered to be anemic (diseased).

Among a sample of 200 ducks trapped at a lead poisoning hot-spot, we find 40 birds that meet the criteria for lead poisoning and 38 birds that are anemic. Of the 38 anemic birds, 32 also fit the definition for diagnosis of lead poisoning. One method for analysis of this type of data is through the use of a 2 × 2 contingency table:

	Cases (anemic)	Non-cases (not anemic)	
Exposed (lead- poisoned)	32 (a)	8 (b)	40 (a + b)
Not exposed (not lead-poisoned	6 (c)	154 (d)	160 (c + d)
	38 (a + c)	162 (b + d)	200

Once the data are arranged in this format, there are several methods by which the strength of association between lead and anemia can be measured. One measure used commonly in cross-sectional studies is calculation of **relative risk** (RR). This is the ratio of the rate of occurrence of disease in those exposed to the risk factor to the rate of occurrence of disease in those not exposed. If there is no association between the factor and the disease, RR should = 1. If RR = >1, the size of the value of RR is directly related to the strength of association between the two variables. If RR = <1, there is a negative association between the factors, i.e., the factor may reduce the occurrence of the disease. In this example:

$$\mathrm{RR} = \frac{\text{prevalence of anemia in ducks with lead poisoning}}{\text{prevalence of anemia in ducks without lead poisoning}}$$

$$= \frac{a/a+b}{c/c+d} = \frac{32/40}{6/160} = 20.$$

The risk of being anemic is 20 times greater among lead poisoned birds than in non-lead poisoned birds, indicating a strong association between lead and anemia.

Another ratio that may be calculated is the **odds ratio** (referred to briefly in Chap. 2). Odds ratio is the probability (or odds) that a case (an anemic bird) has been exposed to lead, divided by the probability that a control (non-anemic) bird has been exposed:

$$\frac{\dfrac{a/(a+c)}{c/(a+c)}}{\dfrac{b/(b+d)}{d/(b+d)}} = \frac{a/c}{b/d} = \frac{ad}{bc} = \frac{32 \times 154}{6 \times 8} = 102.7$$

In this sample of birds, the association between exposure to lead and anemia is obviously strong. When the prevalence of the disease within the population is low (<5%), odds ratio is similar to RR. Comparison could also be made using a more conventional method, such as chi-square (X^2), in which case:

$$X^2 = \frac{n\,([ad\text{-}bd] - n/2)^2}{(a+b)(c+d)(a+c)(b+d)} \quad \text{with one degree of freedom.}$$

The value for significance at the 5% level is 3.84. The calculated value in this example is 116.4 and since this exceeds 3.84, there is less than a 5% probability that a difference as large as observed would occur due to sampling error. We can reject our hypothesis and infer that there is an association between lead poisoning and anemia. Because both variables were measured on a continuous scale, one could also use regression analysis in this instance.

An advantage of a prevalence study is that one is comparing samples drawn from a single population and all diseased and non-diseased individuals in the population should theoretically have an equal chance of being included in the sample. In real life, as has been pointed out elsewhere, this assumption is probably seldom valid. For instance, many of the most severely lead poisoned birds are probably unavailable for capture, while some birds that have been exposed to smaller amounts of lead might be unusually susceptible to the method of capture. A disadvantage of prevalence studies is the large total sample size that may have to be examined. The sample size required is inversely related to the prevalence of the disease, or other factor under consideration, in the population. If the prevalence rate is very low, a large number of individuals must be included in the sample to ensure that the sample contains sufficient diseased individuals for comparison. As in every type of study, selection and collection of an appropriate sample is important; this subject will be discussed more in the following chapter.

6.2.2.2 Case-control method

In many instances, a second type of analytical study, the **case-control** technique, is more efficient than the prevalence survey, because individuals with a special characteristic such as the presence of disease are specifically chosen for inclusion in the study. The basic method in such studies is to identify the association to be measured, e.g., the relationship between lead and anemia,

and then to identify an appropriate number of individuals that have one of the features to be studied. These individuals are the **cases**, and often these are chosen on the basis of presence of the disease. Another group of individuals that do not have this factor are then identified and used as **controls** for comparison.

We can apply this technique to the example of anemia and lead poisoning. Measurement of the concentration of lead in blood is relatively costly, whereas anemia can be detected in the field by centrifuging a small volume of blood to determine PCV using a simple microhematocrit centrifuge. The prevalence survey was wasteful because blood from 200 ducks was analyzed for lead content, of which only 38 of the ducks were anemic. An alternative would be to **screen** a large group of birds, using the inexpensive PCV measurement, to select a sub-sample of birds with anemia (the cases) from within this group for study. An appropriate number of birds without anemia (controls) could also be chosen and lead analysis would then be done only on blood from the ducks in these two groups. In the prevalence study, 200 lead analyses were done, including 38 anemic birds. A case-control study that included 38 anemic birds and an equal number of non-anemic birds would require analysis of only 76 samples, for a substantial financial saving. This relative advantage of case-control studies over prevalence studies becomes progressively greater as the prevalence of the disease in the population declines.

The most difficult part of a case-control study lies in choosing appropriate controls. Ideally, controls should differ from cases only in the single factor under consideration but it is seldom possible to match cases and controls this completely. In choosing controls, three basic decisions must be made: (i) source of controls, (ii) selection of controls from within the source, and (iii) number of controls. The source of controls is obvious in some situations, e.g., if interested in the effect of a water-borne pollutant on animals using river water, one might sample downstream from the source of contamination for cases and above the source for controls. Alternatively, one might sample from two similar watersheds, one of which was contaminated. In other situations the choice is more difficult. Assume that we are interested in the association between renal lesions and antibodies to *Leptospira* spp. in skunks. One source of specimens might be skunks submitted for necropsy to a diagnostic laboratory. These animals would be submitted for many reasons, but primarily to determine the nature of some observed illness. Cases, i.e., animals with renal disease, could be selected from among the animals submitted to the laboratory. The advantage of this source of specimens is that little cost would be incurred in collecting the animals. Several sources might be considered for control animals, including animals without renal disease from among those submitted to the laboratory. However, this sample should be questioned, as the animals have already been selected from the population because of the presence of illness. Hence, they are not likely to be representative of the population. Other sources of controls might be nuisance animals collected by pest-control operators, or from a sample of skunks collected specifically for

the study by trapping. Each of these sources is subject to bias and arguments could be mounted in favor or against the suitability of each. Identification of the biases, and their probable effect on the data, is the most important consideration in choosing the source. In some circumstances, one might choose to use more than one source for controls. If similar results are obtained using control groups chosen from different sources, this is evidence that the observed association is true, whereas if the estimates of risk are different, one should suspect that one or both of the control groups is biased, and the source of bias should be investigated (this should not be taken as condoning trying various control groups until one is found that yields the desired result and then reporting only this result!).

One source of controls that may be appropriate for certain investigations is specimens collected at a time different from that of the cases. Reference collections and various types of specimen banks are particularly valuable in this regard. For example, much of our knowledge of the effects of chlorinated hydrocarbons on the thickness of eggshells is the result of case-control type comparisons between eggs collected from contemporary birds that had been exposed to these agents, and eggs collected in the pre-insecticide era held as museum specimens. Similarly, the concentration of mercury in the feathers of contemporary birds has been compared with that in feathers from museum specimens collected prior to industrialization and to the use of mercurial seed-dressing agents. The latter comparison clearly documented temporal changes associated with this risk factor (Berg et al. 1966).

Selection of individual controls from within the source usually involves sampling and, in most instances, also involves some degree of matching between case and control samples. Careful selection and matching of cases with controls maximizes the information available from a comparison, because it reduces differences between groups in variables other than the one being considered. Some variables, such as sex, age and species, are so obvious that researchers should not need to be reminded of the need for their consideration in matching. In some studies, it may be advantageous to pair individual cases and controls, e.g., a 5-year-old female deer from aspen habitat (the case) would be matched with a control animal of the same species, age and sex collected from a similar habitat (providing that the relationship between these variables and the disease is not under consideration). Overmatching, in which case and control are matched for some determinant that is important in the disease may occur and result in a falsely low estimate of relative risk.

The number of controls required in a study depends on the ease of collection, cost of analysis, and the statistical methods used. In general, at least as many controls as cases should be examined. Many statistical methods benefit from having equal-sized samples and this is a requirement for some techniques. In some circumstances, it may be desirable to analyze more controls than cases to reduce variation within the sample. The same types of statistical analyses used for prevalence studies are applicable to case-control studies and RR and the odds ratio may be calculated.

6.2.2.3 Cohort studies

Prevalence surveys and case-control studies deal with disease existing at the time of the study. The third type of analytical study, **incidence** or **cohort** studies is concerned with **development** of disease in a group of animals. Often the animals studied are free of the disease at the initiation of the study. The term cohort describes a group of individuals who have something in common at the time they are assembled as a group and who are then followed for a period of time to see what happens to them. Cohort studies are potentially useful because they are a more direct method of measuring the risk associated with a disease factor or agent. These studies can be done in two ways. A group of animals can be assembled in the present and followed into the future (a **concurrent cohort study**) or a group can be identified from past records and followed to the present (an **historical cohort study**). In general, cohort studies require the ability to monitor both the occurrence of disease and exposure to one or more risk factors in individual animals over time. Exposure to the risk factor may occur prior to, at the time of, or after the beginning of the study. The occurrence of disease can be monitored in many ways such as through periodic observation or examination of the animals, or through collection and analysis of blood, feces or other specimens at specified intervals.

Bird banding and other mark/recapture techniques that are used to measure mortality or survival represent a form of cohort study. A cohort of hatch-year mallards that is banded in one year and has their subsequent fate monitored through band returns represents a type of concurrent cohort study. A historical cohort study might involve examining band returns to date from all blue-winged teal banded in 1980. In such studies, death, monitored remotely through band returns, is the only measure of disease and the risk factor under study is the summation of all causes of mortality. Through such studies, comparisons can be made among cohorts. For example, the survival rate of birds of the same species banded in the same year in different flyways and, presumably, exposed to different risk factors could be compared. This might be a technique for monitoring the effect of replacement of lead shot by non-toxic steel shot, assuming that the level of usage of steel shot is different among flyways, and can be quantified (however, it would be difficult to separate the effects of lead from those of all other causes of mortality).

The basic requirement for any cohort study is the ability to follow the animals through time. The longer it takes for disease to develop following exposure to the risk factor, the longer the cohort must be followed. Cohort studies have received relatively little use in the study of diseases of wildlife to date because of the difficulty in finding and following individuals. Studies of neonatal mortality of ungulates (Ballard et al. 1981; Nelson and Woolf 1987) and of mortality in a variety of other species (Schultz 1980; Sargeant et al. 1982; Nicholson and Hill 1984; Evelsizer 2002) using radiotelemetry have many characteristics of cohort studies. Burns et al. (2005) used a cohort study design to assess the effects of bot flies on white-footed mice.

Cohort study design can also be used to measure the effect of experimentally applied risk factors. Studies of the effects of ingested lead pellets on duck survival are examples of a form of cohort study (Bellrose 1959; Deuel 1985). In these studies, the risk factor (lead pellets) was artificially applied, so the studies are, in reality, experimental rather than observational; however, these studies are useful for explanation of methodology and for explaining some limitations of this type of study. In both studies a large number of wild ducks was trapped and banded. Lead shot were administered orally to approximately half of the birds before they were released into the wild. The fate of the birds was then monitored through band returns (note that this study has features of a field trial). The cohorts for comparison were the group of ducks that was exposed to the risk factor (lead) and the group that was not exposed. The assumption in both studies was that band returns accurately measured mortality.

A problem in both of these studies was related to having an unequivocal definition of the groups. Bellrose (1959) examined birds with a fluoroscope to detect previously ingested lead pellets prior to the onset of the study. This technique does not identify all birds exposed to lead but was the most acceptable method for measuring lead exposure at the time. No attempt was made to ensure that the birds in the California study were free of lead at the onset of the trial (Deuel 1985). Thus, some birds in both the non-exposed and the exposed group in each study may have been exposed to lead, and the proportion of such birds in each group was unknown. The inherent assumption was that any such exposure was the same in the two groups and that any effect was associated with the administered dose of lead.

Data in Table 6.3 were taken from Bellrose (1959) to demonstrate calculation of RR of mortality occurring in association with exposure to the administered dose of lead. Bellrose used the term "*relative hunting vulnerability*" but it was calculated in the same way as RR.

Table 6.3 Band recovery within the season of banding from wild mallards exposed to different numbers of lead pellets. The number of birds banded in the 0- and 1-pellet groups is a total for 3 years, whereas all of the birds in the 2- and 4-pellet groups were banded during a single year. Data are from Bellrose (1959)

Number of pellets administered	Number banded	Number recovered
0 (non-exposed)	1,456	116
1	1,455	161
2	392	95
4	504	99

If the data in Table 6.3 for ducks receiving either 0 or 1 pellet are arranged in a 2 × 2 table:

	Recovered	Not recovered	
Exposed (1 pellet)	161 (a)	1,294 (b)	1,455 (a + b)
Not exposed	116 (c)	1,340 (d)	1,456 (c + d)

RR associated with 1 pellet is $\frac{a/a+b}{c/c+d} = \frac{161/1445}{116/1456} = 1.38$

The RR of being killed by a hunter was 1.38 times greater for ducks receiving one pellet than for ducks not exposed to additional lead. The RR for ducks given two and four pellets was 1.89, and 2.12, respectively, compared to ducks not exposed to additional lead. This indicates a dose/effect interaction. Deuel (1985) used a different approach and monitored band returns over the 5 years following experimental exposure to lead. No significant difference was found in the rate of band returns between birds given two lead pellets and those not given any lead. The calculated RR, using these data, was 1.

A study of bovine tuberculosis in European badgers (Cheeseman et al. 1988) illustrates the value of a cohort study for determining the evolution of a disease within a population and in individual animals. The spatial distribution of individual groups of badgers was determined and fecal samples were collected from each group biweekly to monitor occurrence and spread of infection among groups within the population. Individual badgers within groups with fecal samples positive for *Mycobacterium bovis* were captured and examined clinically at 3-month intervals. During the initial 5 years of the study, the spread of infection among groups was slow and restricted, and mortality related to *M. bovis* was low, with some infected badgers surviving ≥ 22 months. There was evidence of both horizontal and vertical transmission within groups and no relationship was apparent between population density and prevalence of infection.

Weigler et al. (1988) followed individual koalas naturally infected with *Chlamydophila psittaci* for 24 weeks and observed the development and/or resolution of clinical disease in the animals. This provided an understanding of the course and significance of this infection that could not have been attained by other methods, such as cross-sectional sampling.

Brown et al. (1990) used a cohort design to study the effect of vaccination for rabies on the survival of adult raccoons in an area where rabies was enzootic. Equal numbers of vaccinated and unvaccinated wild-caught raccoons were fitted with radios and released (note that this study is in reality a field trial). The animals were monitored for several months but no difference in survival was detected between the two groups.

A disadvantage of cohort studies for the study of disease in wild animals is the large number of animals that may be required, because of the difficulty in following subjects. For instance, a minimum of 7,946 female pintails would be required to provide an 80% chance of detecting a 20% difference in recovery between lead-dosed and non-dosed birds, because of the low rate of band

returns (Deuel 1985). It is probably not surprising that no difference in survival between lead-dosed and non-exposed birds was detected in that study. Despite the limitations, cohort studies deserve consideration in situations where animals can be monitored regularly and the development of disease can be measured. The technique is particularly suitable for situations in which animals are predictably available for periodic reassessment. A prime example are colony nesting birds, where many individuals are available, and a cohort can be followed through the nestling period and into subsequent years, because of their nest site fidelity. For instance, Hannsen et al. (2004) measured the effect of vaccination with non-pathogenic antigens on survival of nesting common eider females and Wimsatt et al. (2005) used a cohort study design to measure the effects of anesthesia and blood sampling on the survival of big brown bats. Use of radiotelemetry to relocate animals may extend the use of cohort studies to a wide variety of other disease situations. Evelsizer (2002) used a cohort design to compare survival of radio-marked mallards on lakes where bird carcasses were and were not removed during botulism outbreaks.

Observational studies of disease may be either **retrospective** or **prospective** in nature. The major difference between the two types relates to the timing of data collection. Retrospective studies use data recorded in the past, i.e., before the start of the study, while prospective studies involve the active collection of information for the specific purpose of the study. Retrospective analysis is dependent upon the quality of data collected in the past. A common problem is that because the information was not collected specifically for the study, portions of data may be missing or recorded in a manner inappropriate for the desired review. The lack of detailed records of disease in wild animals has limited the use of retrospective analysis; however, such analyses may be an efficient method for gathering information, particularly on diseases that occur infrequently. For example, about once each year, a pronghorn antelope found dead or dying with severe skin lesions has been submitted to our diagnostic laboratory. These cases have been handled routinely and the bacterium *Arcanobacterium pyogenes* has been isolated from the lesions in almost all instances. Each of these cases was an interesting (but seemingly unrelated) curiosity at the time it was examined. However, when records of disease conditions recognized in pronghorns were reviewed, it was obvious that these cases fit together to form a distinct pattern. This pattern or syndrome was characterized by a distinct sexual prevalence (all cases were in males), seasonality (autumn–early winter), distinctive pathologic lesions (necrotizing purulent dermatitis confined to, or most severe on, the head and neck), and presence of *A. pyogenes*. The collected data allow description of this syndrome and formulation of a hypothesis that the disease is associated with wounds suffered by males during the rut, and that pronghorns may have poor resistance to this common bacterium. This retrospective review of the available records could provide a basis for further analytic study. Davidson et al. (1990) used a similar method to study brain abscesses in white-tailed deer. As data collections become more available in future, retrospective studies will become increasingly useful for the study of disease in wild animals. Use of

historical materials, such as museum specimens of eggs and bird skins, as controls for retrospective studies was mentioned earlier. A remarkable data set based on $>2{,}000$ clutches of eggs collected from British sparrowhawks between 1870 and 1990 was used to demonstrate that (i) eggshell thinning coincided with the introduction of DDT, and (ii) shell thickness increased as use of the pesticide was restricted and then banned (Newton 1998).

Cohort studies may be historical in nature. These represent a form of retrospective analysis in which individuals with a particular disease are traced back in time to examine their exposure to various risk factors in the past. This type of study has proven particularly valuable for the study of rare diseases in humans but requires an accurate historical record on the individual, something that is seldom available for wild animals. However, this type of study can be used in wildlife for investigating diseases that leave recognizable traces in the animal. For example, antibody in serum is evidence of past exposure to a disease agent and lead accumulated in bone or mercury in plumage are evidence of exposure to these heavy metals. Similarly, analysis of elements in tissue, such as copper in hair, may reveal the availability of this nutrient to the animal during the period that the hair was growing. Thus, if one was interested in a neurologic disease in birds, it might be possible to select individual birds affected with the disease as a cohort and measure their past exposure to certain viruses by looking for antibody in their serum, and to lead and mercury by analysis of bone and feathers. Findings in these birds could be compared to those from a group of similar birds that did not have the disease. This example blends the characteristics of cohort and case-control studies, illustrating the overlap that may occur among methods.

In prospective studies, the process of information collection can be planned carefully to fulfill specific objectives of the study and, in most instances, the period of data collection is relatively short. This often requires an intense effort but should result in the collection of information of uniform quality. Some diseases occur so infrequently that it is impossible to amass sufficient data over a short period of time and as the period of data collection lengthens problems of non-uniformity of data become more severe. Information collected over a period of years may suffer from many of the same shortfalls described for retrospective studies. This is a problem particularly for investigators working in diagnostic laboratories or disease investigation units. These individuals have a unique opportunity to see and handle diseased animals but their primary responsibility is to investigate each new problem as it arises, rather than to do in-depth research on any one problem. Information collected from the routine activities of such laboratories and individuals may be valuable for retrospective analysis but often suffers from the deficiencies mentioned earlier. There is the risk in any extended study that short-term trends related to a disease may become obscured by long-term trends in population density or abundance unrelated to the disease under study.

One method of combining the benefits of planned data collection with the intermittent availability of specimens and information is an **opportunistic**

prospective study. As an example, our diagnostic laboratory receives a small number of beaver each year for necropsy. Among these animals there have been several with severe degenerative joint disease. A retrospective review of records on these cases indicated that in most instances, the joint lesions were considered to be the major disease process, although the ultimate cause of death was often starvation or misadventure. The animals were usually described as 'aged' in the records, but their actual age had not been determined and, in a few instances, the sex had not been recorded. Based on the observations, one might suspect that debilitating degenerative joint disease is an age-related phenomenon of unknown prevalence in beaver. To investigate this phenomenon further would require additional beaver for examination. One way to proceed might be to collect a large sample of beaver, perhaps from trappers, and do a cross-sectional survey to determine the frequency of occurrence of the disease in various age groups. However, the prevalence of the condition in the population is probably quite low, so that a very large sample would be required, (minimum sample size will be discussed in Chap. 7), and this would require a major research effort.

An alternative would be to do a prospective study using all beaver submitted to the laboratory in the future as a sample, which would be available at little cost, and to collect uniform information related to joint disease from each beaver (the obvious disadvantage, as mentioned earlier, is that such animals may not be representative of the population). For this type of study, we have found that a specific protocol form (usually 1–2 pages) should be designed. The protocol contains a brief statement of the rationale and objectives of the study, a detailed definition of the disease under consideration, together with specific instructions on the information and specimens to be collected. The latter information is arranged in checklist format so that omissions are obvious. Thus, in a study of the association between age and the occurrence of joint disease in beaver, we might provide space on the form for recording weight, sex, and certain body measurements of each animal. The protocol would also specify that a specific tooth be removed and sectioned for aging by cementum annuli examination; that specific bones and joints be examined with lesions being described in a specific manner and photographed; and that certain specimens, perhaps synovial membranes, would be collected for histology.

An advantage of this system of data collection is that different individuals, who may be working in the laboratory, can follow the protocol and collect data in a uniform manner. We have found that several small research projects of this type can be done simultaneously without disrupting the normal diagnostic function of the laboratory unduly. Thus, presently in the Canadian Cooperative Wildlife Health Centre laboratories we have separate protocols for collecting tissues from raptors for lead and anticoagulant analysis, for collecting tissues from some piscivorous birds for mercury analysis, for examining the spinal column of raptors for fractures, as well as examining all wild ungulates for chronic wasting disease. Each study is activated only when an appropriate specimen became available.

The sequence in the investigation of a particular disease is usually, first, recognition of its occurrence, followed by descriptive studies that define the disease and provide the information needed for formulation of hypotheses. Once a hypothesis has been developed, the investigator can then choose among the experimental and observational techniques available for testing it. In general, experimental studies are more rigorous and may be subject to less bias than are observational studies. Experimental studies can be replicated, if necessary, whereas it is impossible to replicate observational studies exactly. However, the results of observational studies may be more directly applicable to a field situation, since they measure naturally occurring, rather than contrived, disease occurrences. Observational studies also may be the only method feasible for situations where the conditions that prevail during a disease occurrence cannot be reproduced experimentally or where experimental studies are impossible, such as in some parks or when dealing with endangered species. The basic techniques described in this chapter can be modified to fit almost any situation. Even elaborate techniques, such as discriminant or multivariate analysis, in which a myriad of environmental factors are measured in relation to disease occurrence, are extensions of simple observational methods.

A potentially rewarding method, which has received relatively little attention, is the combination of experimental and cohort techniques. More than 70 years ago, Aldo Leopold (1939) recognized that observational and correlational studies have limitations for understanding disease. More recently others have expressed the need for experimental perturbation or manipulation to extend our knowledge of disease processes in wild populations (Tompkins et al. 2001). The manipulation might consist of either adding or removing a disease agent and then studying the effect on the population. The study by Bellrose (1959) of mortality associated with lead ingestion by ducks was an early example of adding a disease agent. Other examples also deal with the effects of toxicants on birds. Gilman et al. (1978) extracted organochlorine contaminants from gull eggs in the contaminated environment of Lake Ontario and injected this material into uncontaminated eggs in a colony in New Brunswick in an attempt to separate the direct effects of the toxicants from other factors that may have been operating in the contaminated environment. The cohort for study consisted of eggs in the New Brunswick colony, some of which were exposed to the risk factor (the contaminants) and some of which were not. The eggs were incubated and hatched by the natural parents and embryo and chick mortality were monitored and compared. McEwen and Brown (1966) used this method to determine the effect of two pesticides on sharp-tailed grouse. Wild adult male grouse were trapped, fitted with radio-transmitters, given a single oral dose of one of the pesticides or lactose (control birds) and then released. Survival and behavior of the birds was monitored by radiotelemetry and direct observation on the breeding grounds. The lethal dose of pesticide for these birds was found to be similar to that determined in prior experiments using penned birds. However, changes in social hierarchy, breeding behavior, and vulnerability to predators

detected in the free-ranging birds exposed to sublethal doses of pesticide had not been detected in earlier trials with penned birds.

This method may also be appropriate for certain infectious diseases. Samson et al. (1987) exposed some lambs within a free-ranging bighorn sheep herd to a known number of larvae of *Protostrongylus* spp. lungworms and then monitored the health of the artificially exposed (and of unexposed) members of the lamb cohort by measuring larvae in the feces, clinical signs and survival over the subsequent winter. The advantage of this type of study is that exposure to the risk factor is controlled, as in an experiment, while other variables that may be important in the natural disease are allowed to occur in a manner not possible in the laboratory. Conversely, it may be possible to remove or reduce the effect of a disease agent on a cohort within the population. This has been done by using anthelmintics to study the effects of cecal nematodes on red grouse (Hudson et al. 1992), abomasal worms on Soay sheep (Gulland 1992), intestinal nematodes on snowshoe hares (Ives and Murray 1997), gastrointestinal parasites in yellow-necked mice (Ferrari et al. 2004), and fleas on Richardson's ground squirrels (Jardine et al. 2006) and by experimental supplementation with a nutrient (selenium) in mule deer (Flueck 1994). In each of these situations information was discovered that could not have been identified by observation alone.

6.3 Use of indicator or sentinel species

In some situations it may be advantageous to use a species other than the one of direct concern to collect information about disease. One reason for doing this may be in circumstances in which it is impossible to adequately sample the main species, because it is rare or endangered. Northern bobwhites were used as a surrogate to investigate the presence and prevalence of disease agents on range occupied by the endangered Attwater's prairie chicken (Purvis et al. 1998) and black-footed ferret X Siberian polecat hybrids and domestic ferrets were used as a surrogate in developing disease control measures for endangered black-footed ferrets (Williams et al. 1995). Another reason for using a surrogate is that it may be much easier to work with the surrogate than the species of concern, e.g., domestic chickens have been used as sentinel birds for western equine encephalomyelitis virus for many years in Saskatchewan because it is much easier to put out small flocks of chickens around the province that can be bled for serology periodically, than it is to capture an equivalent number of wild birds. A third reason for using a sentinel species occurs in situations in which a scavenging or carnivorous species screens a large number of the species of concern (which is at a lower trophic level). Measuring evidence of disease in the carnivore/scavenger provides an index to the relative frequency of occurrence of disease in the primary species of concern. Wild carnivores (Gage and Montenieri 1994) and domestic dogs and cats (Leighton et al. 2001) have been used to monitor disease, including

plague, in small rodents. A relatively small sample of carnivores yields information comparable to that obtained by trapping a large number of rodents. A further advantage is that carnivores are longer lived and, hence, available for sampling over a more extended time period than the rodents. Nugent et al. (2002) proposed that feral pigs marked with a radio-transmitter prior to release are an efficient and sensitive sentinel for detecting the presence of bovine tuberculosis in brushtail possums in areas of New Zealand. Of 17 pigs released in an area with a low density of possums, 15 were recovered > 2 months later and all had become infected with *M. bovis*.

6.4 Summary

- A hypothesis is a proposition (set forth as an explanation for the occurrence of a phenomenon) that can be tested.
- The basis for scientific investigation is the collection of information to formulate and test hypotheses.
- Experimental methods measure the effect of manipulations caused by the investigator; observational methods collect information about naturally occurring events.
- There are three sub-types of experimental techniques that differ in the way subjects are chosen for inclusion in the study, in the amount of control that the investigator has over variables, and in the method used to assess changes in other variables.
- Descriptive observational studies dominate the early phase of most investigations and involve the description of disease-related events in the population. Associations among factors may be observed but the strength of the associations is not measured.
- Analytical observational techniques are of three basic types: prevalence surveys, case:control studies, and incidence or cohort studies; all attempt to explain the nature of relationships among various factors and to measure the strength of associations.
- Prevalence surveys and case:control studies deal with disease existing at the time of the study; incidence studies are concerned with the development of disease over time.
- Observational studies may be retrospective, using existing data, or prospective with collection of new information.
- The investigation of a disease may require application of several different techniques singly or in combination. The methods that have been used to study wildlife diseases often have elements of several types of technique.
- Experimental manipulation or perturbation, for example by adding or removing a disease agent from some animals in a population, may be very useful for detecting and measuring population-level effects of disease.
- In some situations it may be advantageous to collect data about disease using another species as a surrogate, indicator, or sentinel for disease in the species of primary concern.

7 Samples, sampling and sample collection

> "*The proper balance lies somewhere between the attitude that if you need statistics your results aren't any good, and the attitude of the compulsive referee who demanded a statistical test to prove significance when all 1000 nematodes chose chamber A rather than chamber B given a choice and equal access*"
>
> (Green 1979)

In disease investigation, the term sample is used in two different ways: as a synonym for specimen, as in blood sample, and in the statistical sense of a sub-collection or sub-set of units drawn from the population. **Collection and analysis of samples is the basis of investigation, and the validity of the results and conclusions of any study is totally dependent on the quality of the samples collected**. Samples of the specimen and statistical types will be discussed separately, later in this chapter; a few general principles will be considered first.

Every study should follow a logical progression from definition of its purpose, through identification of appropriate questions, formulation of hypotheses to be tested, to the selection of appropriate samples and methods. The hypothesis to be tested determines the type of samples to be collected and the appropriate methods of collection and analysis, rather than the reverse being true. An investigator who collects a large number of samples, or a mass of data, without having a clearly-defined hypothesis or a plan for analysis, deserves the unpleasant treatment he is likely to receive when he ultimately has to ask for help. Before any samples are collected the investigator should:

- ensure that the hypothesis being tested is stated clearly (a good way to do this is to try it out on knowledgeable and critical colleagues; if it makes sense to them it is probably satisfactory).
- identify the individuals who will analyze the samples or data and, in consultation with them, choose the most appropriate samples and methods of analysis, including the statistical tests to be used.
- determine if controls are required, and if so identify them.
- determine the number of samples that will be required to test the hypothesis with an acceptable degree of precision and confidence.

After these steps have been completed, a preliminary or trial collection should be done to ensure that the proposed plan is feasible in the real world, and to determine the efficiency of the sampling devices and techniques that are to be used. A trial run may delay the project somewhat and seem to be a waste of time but almost invariably will end up saving time and money.

7.1 Error

The single greatest concern during any type of sampling is to ensure that the samples collected are representative of the actual situation or population from which they are drawn. If the sample is not representative, the results of any analysis will not reflect the true situation in the population. Two types of error, **random error** and **error due to bias**, may occur during sampling and can affect the representativeness of samples. Random error, often called sampling error, arises because we only examine a portion of the population. If we were able to examine the entire population, random error would be zero. Random error is the less serious of the two types. Although it results in decreased precision and sensitivity, this can usually be dealt with by statistical means and by increasing the sample size.

Errors caused by bias are much more serious because there is a systematic distortion as a result of the sampling procedure. Statistical methods often cannot deal with this type of systematic error. Most bias falls into one of three categories: selection, measurement, or confounding bias, although there may be considerable overlap.

7.1.1 Selection bias

Selection bias refers to a distortion that occurs from the way in which subjects are selected for study. Hunter-killed animals often are used in disease studies but whether or not such samples are representative should always be questioned. For example, mallards killed by hunters shooting over decoys weighed significantly less than did mallards collected at the same time on roosting areas (Greenwood et al. 1986) and mallards in poor body condition at the time of banding in autumn had a greater probability of being killed during the subsequent hunting season than did birds in good condition (Hepp et al. 1986). Taken together, these two studies suggest that a sample of hunter-killed mallards is likely to be biased in favor of birds in poor body condition. Similarly, hunters selectively shoot males of those species of ducks in which the male is more brightly colored than the female, whereas equal numbers of males and females of species without obvious plumage dichromatism are shot (Metz and Ankney 1991) and deer with chronic wasting disease are likely to be hit by cars (Krumm et al. 2005). Selection bias also occurs in

other methods of sample collection. Mineau and Peakall (1987) reviewed methods for assessing the effect of forest sprays on birds. They concluded that in sampling birds after spraying there was a strong bias for selection of birds with low levels of cholinesterase inhibition, i.e., the least severely affected individuals in the population. Birds with more severe impairment hid from the investigators or were less visible and so were less likely to be included in the sample. This selection bias could lead to serious underestimation of the effects of the spray. During a multiyear study of the prevalence of feline immunodeficiency virus in feral cats, Courchamp et al. (2000) sampled approximately 60% of the individuals in the population twice each year. There was a steady decline in the apparent prevalence of the disease during the first 3 years of the study. Because the authors had detailed knowledge of the individuals within the population, they suspected that the data were biased. This occurred as a result of non-random sampling, in that older infected individuals were recaptured less often than other cats. When trapping methods were adjusted so that these individuals were sampled, the apparent prevalence remained at a constant level.

7.1.2 Measurement bias

Measurement bias refers to the distortion that occurs when: (i) the methods of measurement do not reflect the true situation, (ii) measurements are consistently dissimilar among groups, (iii) subjects are misclassified through use of incorrect diagnostic criteria, or (iv) there is unequal diagnostic rigor or surveillance among groups. The first type of measurement bias would occur if one used an incorrectly calibrated instrument, or if there was contamination among samples. For example, inadequately cleaned laboratory equipment may have resulted in contamination of fecal samples from moose with larvae of *Parelaphostrongylus tenuis* and led to an incorrect hypothesis that moose were becoming a suitable host for this parasite (McCollough and Pollard 1993). The second type would occur if one estimated the number of birds in the evening on fields sprayed with pesticide and in the morning on control fields. An example of the third type would occur if one measured prevalence of a parasite by recording occurrence of larvae in the feces of animals, without ensuring that all of the larvae present were of the same species. A technique that is adequate for one disease agent may not be suitable for another agent, e.g., blood samples taken from the veins of birds are used routinely to determine the prevalence of blood parasites. However, samples of blood from the brachial vein underestimated the prevalence of nematode microfilaria but not of *Leucocytozoon lovati* and *Trypanosoma avium* in willow ptarmigan (Holmstad et al. 2003). A common example of measurement bias is the upswing in reporting of a disease, as a result of greater public awareness, that often follows publicity. This type of bias may result in data that suggests that prevalence of a disease has increased, when it actually remained constant.

7.1.3 Confounding bias

Confounding bias occurs when the effect of the factor being investigated is interrelated with the effects of other extraneous variables. This type of bias may result in incorrect conclusions, particularly about cause and effect relationships. If we were to measure residue levels of a cumulative contaminant in tissues of a large sample of deer of all ages, and also examined the deer for evidence of degenerative joint disease, it is probable that there would be a strong statistical correlation between the level of residue and the prevalence of arthritis, i.e., deer with high residue levels also probably would have a high frequency of occurrence of joint lesions. This might lead one to conclude that the contaminant was linked causally to joint disease. In this instance, an underlying or confounding variable, age, results in a spurious association, because both the accumulation of residues and the arthritic condition are age-related. Confounding bias, unlike the other two types, may be correctable at the analysis stage. This could be done in the case of the deer by comparing the prevalence of residues and joint disease on an age-specific basis.

Even if it is impossible to measure the **magnitude** of bias, it is often possible to assess its **direction**. If this is done, one may be able to state that because of the presence of recognized bias, the association observed between factors is either stronger or weaker than the data would suggest. An objective assessment of the assumptions required for every sampling technique, together with preliminary sampling to evaluate the methods, will help in detecting and evaluating the extent of various sources of error. As an example, suppose that we were interested in assessing the association between stress and severity of lungworm infections in bighorn sheep. Field observations suggest that stressed sheep have more lungworms and a testable null hypothesis (H_0) might be that: ‘there is no correlation between the level of stress and the intensity of infection in individual sheep’. We decide to measure cortisol in blood as an index of stress, and the number of lungworm larvae/g of feces as an index of the intensity of parasitism in the sheep. Collection of samples will require capture of the sheep and it is decided to use a drop-net trap that, for logistic reasons, will be located at one easily accessible site on the study area. It is estimated that trapping will have to continue for about 6 months to obtain an adequate sample size. During the planning of a project, an investigator should be assessing the methods on a continuous basis. Much of this can be done in one’s head but it is often useful to construct a list of the specimens and methods that will be used to collect those samples, and then decide the assumptions that are required for each to result in a valid sample. If we do this for the example of the stress:lungworm hypothesis:

Sample	Assumptions
Trapped sheep	– these individuals are representative of the population of sheep in the area.

For this assumption to be valid, every animal in the area must have an equal probability of being captured *or* the variables being studied (cortisol, lungworms) must be distributed in a random fashion among the population and over time. Even a preliminary consideration would suggest that animals in the vicinity of the trap site are more vulnerable to capture than those in inaccessible areas of the range, so that all members of the population are not equally likely to be sampled. Also there is seasonal variation and a clumped (non-random) distribution in the number of larvae shed by bighorn sheep (Uhazy and Holmes 1973).

cortisol in blood
- the concentration of cortisol in blood is proportional to the level of stress experienced by the animal, i.e., it is a true indicator of stress.
- the concentration of cortisol in the blood of trapped sheep is representative of that in free-ranging sheep, i.e., trapping has no effect on cortisol.

The first assumption might be true but would be difficult to confirm without some other independent measure of stress. Blood corticosteroid levels change dramatically with capture and handling, e.g., Franzmann et al. (1975). Thus, the latter assumption is probably invalid. Measuring cortisol in feces might be a way of reducing bias associated with capture (Miller et al. 1991).

larvae in feces - the number of larvae in feces is proportional to the number of lungworms present in the animal, i.e., it is a true indicator of intensity of infection.

Forrester (1971) found that while there was some correlation between the *average* number of larvae shed by members of a group of sheep and the *average* intensity of infection, no such correlation was found in individual sheep. The number of larvae shed in feces is likely to vary seasonally (Arnett et al. 1993; Pelletier et al. 2005), so that samples collected at different periods of year are not comparable.

When samples and inherent assumptions are listed it often is obvious, as in this example, that some or all of the proposed methods are inappropriate. This example also illustrates the constant problem of obtaining a truly random sample of wild animals.

Every method of capture or collection of wild animals (trapping, shooting, netting, road-kills, hunter-killed animals, etc.) is likely to result in a biased sample. Bias may be tolerable in some instances as, for instance, when samples collected in the same manner from two different areas are to be compared. The assumption in this case is that the bias is similar in the samples. Unfortunately, many methods used for selecting random samples in human epidemiologic studies are not practical when working with wild animals. For instance, one cannot assign every animal in the population a number and then select individuals for sampling by choosing numbers from a table of random numbers, as can be done with humans using social insurance numbers. However, one could and should choose trap-sites in this way. In most situations, the wildlife disease investigator has to use the best samples available but must try to identify the presence and direction of bias, and correct for it wherever possible. The study by Courchamp et al. (2000) referred to earlier is an excellent example of this process.

7.2 Collection and analysis of biological specimens

7.2.1 Handling specimens

Because of the specialized nature of medical science, most specimens collected during a disease investigation will be analyzed by someone other than the collector. **The single most important guideline for specimen collection is to consult, in advance, with the person(s) who will do the analyses.** In this consultation, one should establish the number and type of specimens that they are willing to examine, and the precise methods of collection and preservation that they require. There is no universal preservative suitable for all specimens and, in most investigations, the methods used represent some degree of compromise. For example, freezing is a good method of preserving specimens for some tests but it ruins specimens for detailed histologic examination. Fixation of specimens in 10% neutral buffered formalin is suitable for most histologic studies but destroys any possibility of isolating living agents from tissue. The type of container used may invalidate the results of some toxicologic analyses, and dry ice used for cooling specimens may inactivate certain pathogens. General guidelines for collection and preservation of specimens in the field are available in Roffe and Work (2005) but these should be considered as stopgap methods until specific methods are established through consultation with local experts.

Unlabelled specimens, no matter how carefully collected or preserved, have little or no scientific value. Every specimen must be marked in such a manner that the label will not be lost during handling, or by immersion or other contact with liquids. If possible, it is best to take a 'belt and suspenders' approach by labeling each specimen in two different ways. Often this means placing one label, containing a complete set of information, inside the container and a second similar label attached firmly to the outside. Soft copper or aluminum tags, inscribed with a stylus, are close to the ultimate in durability for some types of specimens; linen tags, written on with soft lead pencil or water-proof carbon ink, also are satisfactory. Starch-filled paper tags and non-permanent inks should be avoided, and one should not rely on labels written directly on plastic bags or containers. Labels, as every other part of the methodology, should be tested prior to the main study.

Whenever possible, specimens should be hand-delivered to the laboratory rather than entrusted to a commercial carrier. Perishable specimens should never be shipped by mail and all perishable goods must be packaged to prevent deterioration during transit. It is critical that all specimens and preservatives, including dry ice, conform with the hazardous goods regulations applicable to common carriers. These regulations must be checked in advance of packaging specimens to avoid delays or refusal by the carrier to accept the specimens. If specimens must be shipped, the materials must be packed in a manner so that there is no possibility of spills of liquid contents, and the receiving laboratory should be notified in advance of the shipment

and provided with information including the flight number, expected time of arrival, and the way-bill number. This will reduce delays in delivery and allow tracing of specimens that do not arrive when expected.

Specimens collected during a disease investigation include those from animals and from other parts of the environment, such as water and vegetation. The investigator has to decide whether to do the analysis in his or her own laboratory, or to send specimens to an established laboratory doing the required test. The advantages of doing the analysis in one's own laboratory relate mainly to control over the work and the ability to modify the techniques as required for the project. The disadvantages are the cost of equipment and manpower, lack of experience with the technique resulting in prolonged development time, and inexperience in interpreting the results. The advantages of using an established laboratory are that equipment, technical expertise and experience are in place and, in good laboratories, the quality of analyses is controlled through the regular use of standards. Disadvantages are that specimens from outside may have a low priority, so that analysis is delayed, and the techniques used may not be totally appropriate for specimens related to wild animals. If an established laboratory is used for analysis of specimens, it is advisable to make the appropriate scientists there part of the research team and to involve them in planning the project, rather than simply buying analyses. In this way, tests may be chosen, or modified, that are more appropriate than the standard ones for the particular project.

7.2.2 Interpreting test results

Greiner and Gardner (2000) defined a diagnostic test as "*any device that reduces uncertainty about the state of a disease*". A variety of tests or analyses may be used during the investigation of a disease. In general, it is much easier to collect and analyze specimens than it is to interpret the results of tests. This is particularly true in wild animals, for which baseline data are meagre and normal values are poorly defined. Lack of baseline information has led to a great deal of extrapolation from humans and domestic animals, and to presentation of results without an attempt to determine what, if anything, they mean. Too often test results are accepted at face value without an exploration of their accuracy and precision. It is alarming that more effort has been directed toward analyzing samples than toward the analysis of methods. In many cases, little effort has been made to determine if the method used yields results that are appropriate, valid and meaningful.

I will use tests to detect antibodies as the model for discussion here, because serology provides blatant examples of analysis of samples without consideration of the applicability of the tests used, or of the validity of the results. Many of the same basic problems apply to the use of serodiagnostic tests in domestic animals (Jacobson and Romatowski 1996). Sera from wild

species often are analyzed using techniques and reagents developed for measuring antibodies in humans or livestock without considering peculiarities of the immune response of the wild animal or if the technique works with serum from the wild animal. The arbitrary distinction between positive and negative results developed for a domestic species often is applied without verification of its appropriateness. As an example, a test and cut-off value for detecting antibody to *Toxoplasma gondii* was chosen to study the occurrence of this parasite in lynx on the basis of its sensitivity and specificity in domestic swine (Zarnke et al. 2001). When the applicability of serological techniques developed for livestock to wild animals has been tested, the technique often has proven unreliable. For instance, Thorne et al. (1978) tested four different serologic tests used for detecting brucellosis in cattle on infected elk and concluded that: "*no single serologic test should be relied upon to diagnose brucellosis in elk*". Grimes and Page (1978) tested three methods for detecting antibodies to *Chlamydophila psittaci* in four species of experimentally-infected wild birds. None of the methods detected antibodies reliably in all species, and the methods varied greatly in their specificity among species. Renshaw et al. (1979) evaluated tests, developed for detecting anaplasmosis in cattle, for use in elk and concluded that the tests were satisfactory, but that the methods had to be modified for good results.

Before a serological test is used to detect exposure to a disease in a wild species, the test must be validated. Jacobson and Romatowski (1996) defined a validated assay as one that has been: (i) developed properly through the use of appropriate reagents that are correctly titrated, (ii) subjected to testing of a large number of animals of known disease status, (iii) involves use of proper controls to confirm that each run of the assay is accurate and precise, and (iv) provides estimates of the test's **sensitivity** and **specificity**. The most problematic part of validation in wild animals is part (ii) of this definition, because of the difficulty in identifying animals of known disease status. In some situations, the response and duration of immunity following exposure might be established through experimental infection of that species. In other situations, the disease status may have to be determined by some other test, such as necropsy and bacteriological culture, conducted on a large number of animals. In this situation, necropsy and culture becomes the **gold standard** to which the other test is compared, even though it is unlikely to be 100% accurate. Fitzgerald et al. (2000) illustrate the use of one test (bacterial culture) as a gold standard for evaluating other tests for detecting tuberculosis in deer.

The specificity (the proportion of true negatives correctly identified by the test) and the sensitivity (the proportion of true positives correctly identified by the test) are needed to predict the probability that a positive or negative test result predicts an animal's disease status. The method used to calculate sensitivity and specificity of a test is shown in Table 7.1. The diagnostic sensitivity and specificity of a test describe the performance of the test for a specific reference population under defined conditions (Greiner and Gardner 2000), so that these values should not be expected to be the same in a different population or under different circumstances.

Table 7.1 Calculation of sensitivity and specificity of a test, based on examination of 1,200 animals of which 400 were known to have been exposed to the disease agent and 800 were known not to have been exposed

	Known disease status	
Test result	Exposed	Not exposed
Positive	375 (a, true-positives)	45 (b, false-positives)
Negative	25 (c, false-negatives)	755 (d, true-negative)
	400	800

Sensitivity = a/(a + c) or $\dfrac{\text{true positives}}{\text{true positive + false negatives}}$ $\dfrac{375}{375 + 25} = 93.8$

Sensitivity = d/(d + b) or $\dfrac{\text{true negatives}}{\text{true negatives + false positives}}$ $\dfrac{755}{755 + 45} = 94.4$

In most diseases of wild animals, quantitative aspects of the serologic response to the agent are unknown. When a value is chosen to distinguish between positive and negative results, it is assumed that there are two distinct populations (those with the disease and those without the disease), and that the value chosen represents the division between them. In most infectious diseases there is overlap between the two groups (Fig. 7.1). The threshold or cut-off value that is chosen determines the sensitivity and specificity of the test. For example, if the value indicated by line a in Fig 7.1 was used to distinguish between diseased and non-diseased individuals, the test would be highly sensitive. It should detect a very large proportion of the diseased animals correctly but it would have relatively low specificity, resulting in many false positives. If value b was used as a diagnostic level, the reverse situation would be true. The choice of an appropriate threshold value is a compromise but it should not be an arbitrary decision. Whenever possible it must be based on knowledge of the distribution of values in both diseased and non-diseased

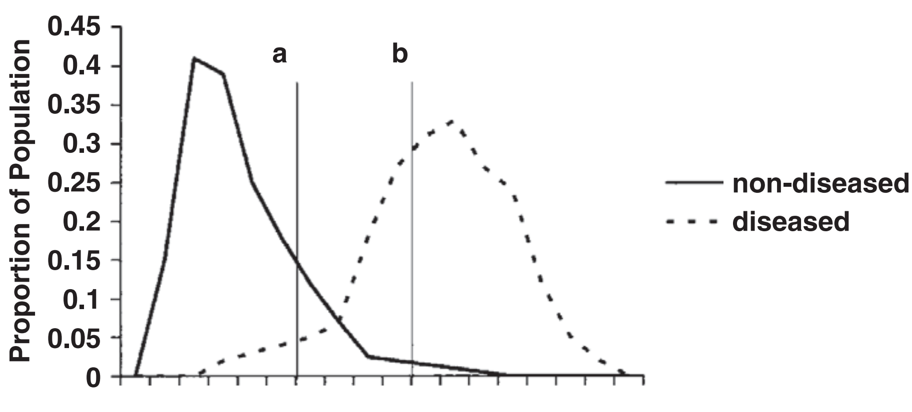

Fig. 7.1 A hypothetical example to show the difficulty in choosing a threshold antibody titre to distinguish affected from non-affected animals. If the value indicated by "a" is used, the test will be highly sensitive but not specific; if value "b" is chosen, the reverse will be true

animals. "*Reference animals of known infection status, numbering in the hundreds, are required to establish strong estimates*" of sensitivity and specificity (Jacobson and Romatowski 1996). Cut-off values can be chosen for a specific purpose, for instance if the purpose of the test was to identify as many infected animals as possible that would be culled for disease control, a cut-off value with a very high sensitivity (such as a) might be chosen, in the knowledge that many false-positive animals will be sacrificed.

For some reason there is a tendency to assume that one wild animal is a suitable model for another related species, whereas similar evidence would not be acceptable in human or veterinary medicine. For example, Shulaw et al. (1986) developed a serologic test to detect antibodies to *Mycobacterium avium* ssp. *paratuberculosis* in white-tailed deer, but determined the validity of the test "*in deer*" by using samples from infected sika and fallow deer. It is doubtful that a test developed to detect disease in humans would be accepted for use in public health circles, if its validity had been established by using squirrel monkeys and baboons! Other variables may affect the validity of serologic surveys. For example, the antibody response to some agents may be weak and ephemeral (Vickers and Hanson 1980; Hathaway et al. 1981), intercurrent disease or malnutrition may make animals immunologically unresponsive, and failure to detect antibodies to a highly fatal disease, such as tularemia, in a susceptible population should not be taken to indicate absence of disease (Omland et al. 1977).

It is important to understand that the prevalence of disease in a population affects the amount of confidence that one can have in the results of a test in individual animals. To illustrate this point, consider the animals described in Table 7.1. At the time of sampling, the prevalence of exposure was 33.3% (400/1,200) and 420 animals tested positive (of which 375 were true positives and 45 were false-positives). We can calculate the positive and negative **predictive value** of the test, i.e., the likelihood that an animal which tested positive actually had been exposed, or that an animal that tested negative actually had not been exposed, respectively (Thrusfield 2005). The predictive value of a positive test result = $a/(a + b)$ or $375/375 + 45 = .89$. In other words, we can be about 89% confidant that an animal that tested positive had been exposed to the disease. Similarly, the predictive value of a negative test = $d/(c + d) = 735/25 + 735 = .97$.

Now assume that disease management has resulted in a decline of the prevalence of disease to 10%. If we were to test another sample of 1,200 animals, assuming the sensitivity and specificity of the test remain the same, the results might be:

	Exposed	Not exposed
Test positive	113	45
Test negative	7	1,035
Total	120	1,080

The absolute number of false-positives has not changed but the predictive value of a positive test result is now 113/113 + 45 = .72 and the predictive value of a negative test = 1,035/7 + 1,035 = .99. If the management program is continued until the actual prevalence of exposure falls to 1%, the results from examining another sample of animals might be:

	Exposed	Not exposed
Test positive	11	45
Test negative	1	1,143
Total	12	1,188

The predictive value of a positive test result is now 11/11 + 45=.20 and the predictive value of a negative test = 1,143/1 + 1,143 = 1.0. At this prevalence, only about one in five animals that tested positive would actually have been exposed. The point of this discussion is that at a low prevalence level even tests that are quite sensitive and specific have a low positive predictive value. If we were using the test to choose animals for culling from this population, 80% of the culled animals would not have been exposed to the disease when the prevalence was 1%. There will still be the same proportion of false-positives even after the disease has been eliminated completely from the population (Thrusfield 2005).

The warning against application of techniques without understanding their inherent usefulness and validity is not limited to serology and applies equally to many other types of study. For example, analysis of brain cholinesterase activity is used widely as a measure of exposure to certain types of insecticide but the normal level of activity, the rate of recovery after poisoning, and the effect of age on activity (Grue et al. 1981) are unknown for most species. Appropriate normal animals of the same species must be collected and handled in the same way as the poisoned individuals for the results of analysis to be meaningful (Hill and Fleming 1982). The decision to extrapolate between species may have serious repercussions. For instance, a decision to allow use of the pesticide carbophenothion as a seed treatment in areas of Scotland used by pink-footed and greylag geese was based on toxicity data derived using Canada geese. The Scottish geese were found to be more susceptible than Canada geese after "*significant proportions of the world populations of these geese had been killed*" (Hart 1990). Specimen handling also may have a great effect on the results of tests (Cohen et al. 1969; Wesson et al. 1979; Hunter and Madin 1978; Walker and Jefferies 1978; Hill and Fleming 1982; Kerr and Pace 1987). For many tests results from another laboratory are not appropriate for use as controls, because of interlaboratory variation.

The important point of this discussion is that each method to be used in a study must be examined critically and tested as completely as possible, using appropriate controls, before the main study is begun. The investigator should

know the answer to the simple questions: (i) exactly what is being measured when I use this test? and (ii) how confident can I be that the results reflect the actual situation in the field?

7.3 Sampling and data collection

This section will not describe specific statistical methods or indicate where certain tests should or should not be used. The intent here instead is to discuss sampling design and choice of samples. The best overall advice one can give in this area is to define the purpose of the investigation as clearly as possible, determine the type of samples that might be collected, and then discuss the project with a statistician, who is cognizant of the problems in working with wild animals, before any sampling is done. "*Proper statistical methods should be used, but the biologically defined objectives should dominate and utilize the statistics, rather than the reverse*" (Green 1979).

7.3.1 Sampling design

The sampling design used in an investigation is determined by the hypothesis to be tested and should attempt to eliminate or control as many unrelated variables as possible. If the study consists of comparing groups, the groups should differ only in the variable defined in the hypothesis, and it is important to ensure that consideration is given to all factors, including space and time. The choice of variables to be measured also is determined by the hypothesis. In most studies, one might collect and compare data on a large number of factors and the problem is usually to decide which factors, from among the many, are appropriate. A useful technique is to list all of the variables that might be examined and then ask: (i) does it measure what I want to know? and (ii) do I have the time, money and analytical resources to collect and measure this variable?

As an example, we were interested in the occurrence of muscle injury in waterfowl during capture for banding. The objective of a preliminary study (Bollinger et al. 1989) was to determine if there was a difference in the amount of muscle injury among birds captured by three methods (bait trap, decoy trap, rocket-net). Because we were interested in the real-life situation, we used birds captured during actual banding operations as the subjects and, to reduce extraneous variability, only adult male mallards were used for comparison. Some variables, such as time of entry into the trap and time in the trap before banding, were beyond control but did not affect the objective because the methods were those used routinely for banding. Many possible measures of injury, including body temperature, blood constituents, electromyography, and examination of muscle biopsies were considered. We

measured the concentration of two enzymes (creatine kinase and aspartate aminotransferase) in blood, because they are sensitive indicators of muscle injury in birds (Franson et al. 1985) and because we had experience in measuring these enzymes in waterfowl. Measurement of the enzymes told us what we wanted to know and their collection under field conditions, and analysis, were possible in terms of budget, time and other considerations. Using this method, we identified differences in the degree of muscle injury suffered by birds trapped by different methods.

After the variables to be measured or assessed have been chosen, the next important step is to select the subjects for study. When little or nothing is known about a disease and only a very rough estimate is desired, it may be acceptable to examine the most conveniently available members of a population; however, if one hopes to attain greater precision, the study population must be chosen with care. In most investigations, some aspect of comparison is required and, ideally, one should compare groups that differ only in the variable under consideration. In real life, everything is usually not equal and an important part of study design is the recognition and control of extraneous variables.

One method to reduce extraneous variability is by **restriction** in choosing subjects. In this process, the subjects accepted for study are restricted to those possessing a specific range of characteristics. Potential subjects that lack these characteristics are excluded. Restriction may make groups more comparable and also helps to increase the efficiency of statistical tests. For example, if one were interested in the prevalence of a disease that only occurred in adult animals, it would be logical to exclude animals that had not reached sexual maturity. In the study of muscle enzymes, we restricted the group to adult male mallards to reduce variability related to species, sex and age differences. We also excluded any bird that had been captured for banding prior to the study, because of the chance that prior experience might alter the response of the bird to capture. Inclusion of such birds might have created a bias. The intent of restriction is to ensure that the subjects do not vary greatly from each other in factors other than those under consideration. The risk in restriction is that overly rigorous exclusion may result in a group that is so narrow that it is not representative of the population. Matching of controls to cases, as described in the preceding chapter, is a very rigorous form of restriction.

It is rarely possible to examine all the suitable individuals in a population, even after restriction, and some type of sampling is usually necessary. The hope is to select a subgroup representative of the population but one must always be cognizant of sampling error or bias in the way the sample is selected.

7.3.1.1 Unrestricted random sampling

If the sample is selected directly from the population, and every individual has an equal chance of being selected, the method is known as simple or unrestricted random sampling. A classic example of this type of sampling is

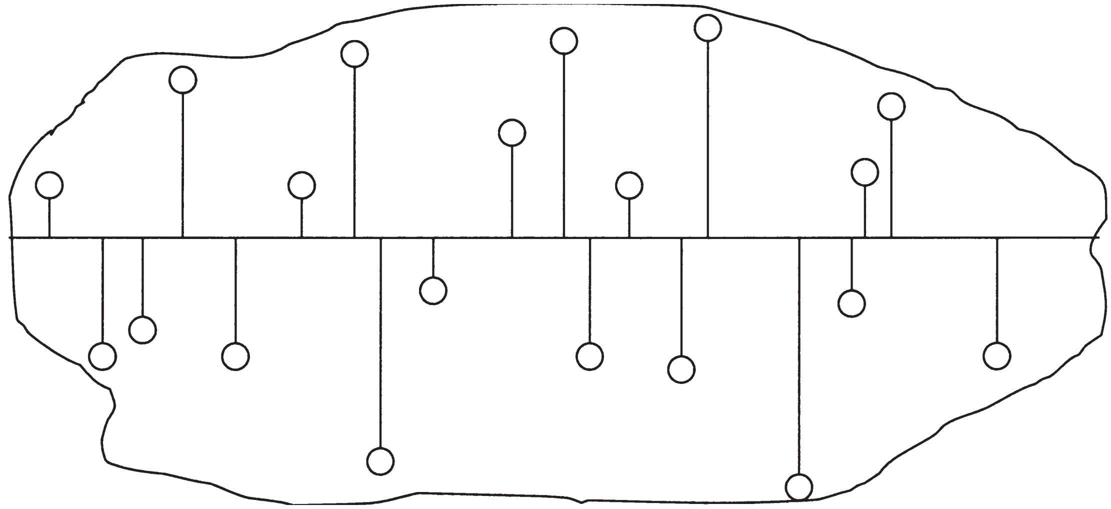

Fig. 7.2 A method for choosing randomly distributed sampling sites in an area with no existing grid system. In this example, a base line was established through the long axis of the area and sampling sites were chosen at various distances perpendicular to the line. The coordinates of each site were chosen by using a table of random numbers to select two numbers. The first number was a distance along the base line and the second was a distance perpendicular to the line at that point. The direction from the line was determined by a coin toss

where it is possible to assign a number to every individual in the population and then to choose individuals for inclusion by selecting numbers using a table of random numbers. Another method of random sampling is to use a table of random numbers to choose sampling sites as illustrated in Fig. 7.2. Fredrickson et al. (1977) used this method to identify coordinates for sample collection sites while sampling marsh soils for lead pellets and we have used it to select sampling sites during studies of the efficacy of searches for carcasses (Wobeser and Wobeser 1992; Philibert et al. 1993). Sampling sites also may be chosen randomly on the basis of latitude and longitude coordinates and then the sites can be found in the field using global positioning (GPS) equipment.

7.3.1.2 Stratified random sampling

If groups within the population vary widely in relevant characteristics, the population may be divided into subgroups or strata and then a random sample maybe selected from within each subgroup. This technique is termed "stratified random sampling". Stratification may be based on factors such as habitat type, age, sex, and species. The assumption is that the variation within strata is less than that within the entire population; if this is not the case, there is no reason for stratification. Stratified random sampling can be used to ensure that the sample contains representatives from each stratum or to ensure that all strata will be fairly represented, i.e., that the proportion of the total sample taken from each subgroup is the same as the proportion of that stratum in the population. Assume that we wish to study a disease in deer and

are dependent on hunter-killed animals for samples. A group of deer examined at hunter check stations was comprised of 43% adult males, 9% adult females, 26% male fawns, and 22% female fawns. Other surveys of the population, believed to be more accurate, indicated that the actual composition of the population was 20% adult males, 24% adult females, and 28% of fawns of each sex. Thus, the hunter-killed sample is not representative of the population. Division of the hunter-killed deer into four strata, based on sex and age, and selection of samples from within these strata, in proportion to their occurrence in the population, might yield a result more representative of the population than that obtained by simple random sampling. In other instances, one may have to take a disproportionately large sample from one stratum to assure that all strata are represented by a sufficiently large sample for comparison. In some situations it may be possible to obtain good, representative samples from some strata and much less satisfactory samples from other strata. When this is the case, one can at least draw inferences for those strata with adequate data, while it may not be possible to do so for the entire population.

7.3.1.3 Cluster sampling

Another method of sampling is to divide the population into subgroups or clusters that are not homogeneous, as they are in strata. Often the unit for division is geographic, such as a county, province, or hectare. A sample of clusters is then randomly selected from within the entire group and either all (or a randomly selected sample) of the individuals within the selected clusters form the sample. This method is often called sampling by stages because of the sequential steps involved. To illustrate the method, assume that we wished to determine the average number of ticks on snowshoe hares in a large study area (20×20 km) during a period of high hare population density. The required sample size was determined in advance to be 200 hares. One method of sampling would be to choose sites for trapping individual hares by simple random sampling, using a table of random numbers to identify coordinates. This would result in single sampling sites randomly spread over the entire 400 km^2. An alternative would be to consider each 1 km^2 area within the study area as a sampling unit (or cluster), and then choose 20 of these randomly, and collect ten hares on each (the assumption here is that the areas chosen are representative of the entire area). The saving in field and travel costs in the latter approach will be obvious to anyone who has attempted field research. However, the savings in cost must be balanced against potential loss of accuracy in the estimate obtained, and there may be bias if the sampling sites are not representative of the entire area. Figure 7.3 illustrates a form of cluster sampling that we used to estimate the number of dead ducks on the shore of a large lake during a severe avian cholera outbreak. An advantage of this method was that measurement of the total shoreline and intensive search of a known proportion of the shoreline were combined into a single field operation.

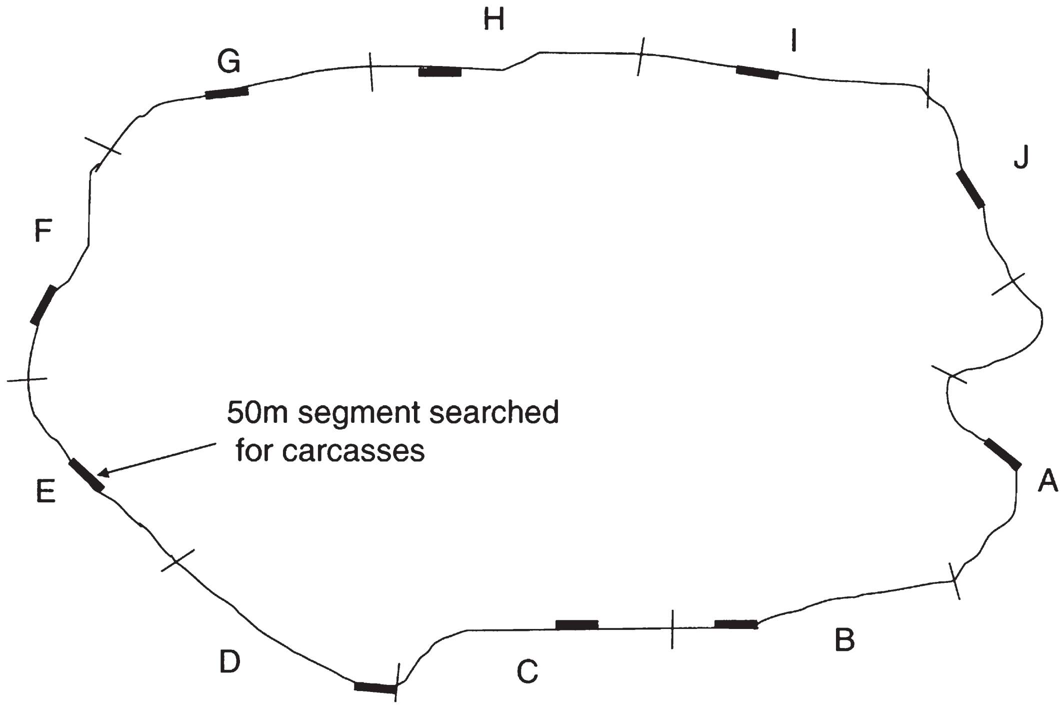

Fig. 7.3 A method of cluster sampling we have used to estimate the number of ducks dead of avian cholera on the shore of a large lake of unknown circumference. It was decided that 10% of the shoreline could be searched. Each 500 m of shoreline was treated as a cluster, within which one 50-m segment was searched for carcasses. Prior to beginning the search, a schedule was constructed containing sufficient 500-m clusters to reach around the expected circumference of the lake and with one 50-m segment within each cluster selected randomly by drawing a number between 1 and 10. Thus, segment 6 was searched in cluster A, segment 2 was searched in cluster B, etc. The starting point was marked and two searchers proceeded around the lake, measuring the circumference and searching the pre-selected segments. The average number of carcasses/50 m was 37.2 (SD = 14.1) from which the estimated number of carcasses, with a 95% confidence interval could be calculated. Our estimate of the number of carcasses was 4,524, with 95% confidence limits of 3,551 to 5,497. The number of carcasses collected during a clean-up 2 days later was approximately 4,900

7.3.1.4 Systematic sampling

Another variation is called systematic sampling, in which individuals are selected by drawing, for example, every sixth or tenth individual from a list or group being examined. We have used this method when confronted with a large sample of dead waterfowl collected during a die-off by an interested resource officer. We did not want to discourage the officer by telling him to collect fewer birds for examination but we could not necropsy all the birds. We examined all the birds externally to determine the species, sex, and age composition of the group and then chose birds for necropsy by sampling. When all the birds were of one species, we performed detailed necropsies on 10 to 20%, using a systematic sampling system. The birds were laid out in a line, and every tenth or fifth bird (depending on the proportion required) was selected for necropsy. When more than one species was involved, stratified

systematic sampling was done, with each species being a stratum, from within which individuals were chosen by the method outlined above. We have also used this technique to collect a representative sample of blood specimens from a very large number of geese being handled for banding. In systematic sampling, it is important to ensure that there is no periodicity in the way that animals are arranged in the list or row, or any recurring pattern in the population from which the sample will be chosen, as this will result in a biased sample.

7.3.1.5 Multistage sampling

Several of the methods described above may be combined into so-called multistage sampling, which may or may not include stratification. As an example, assume that we wished to determine the degree of exposure of ducks across North America to lead, and that we choose to measure the level of lead in wing bones collected from hunter-killed ducks. An initial step might be to restrict the sample by including only wings from ducks killed between October and December of the current year, and to exclude all birds other than adult female mallards (adult female mallards can be identified by wing plumage). By this simple restriction, we have eliminated variation attributable to species, sex, and year of collection and reduced variation related to age (the group of adults will include birds of different ages greater than 1 year). We might then decide to divide the population into strata based on the flyway of origin and, for purposes of comparison, decide to examine an equal number of wings from each flyway. For logistic reasons, it would probably be desirable to collect samples from a few defined but randomly selected areas (clusters) within each of the flyways, rather than randomly from throughout each entire flyway. Clusters might be as large as a province or state, or some smaller unit such as a county. The wings used for analysis could then be chosen by simple random sampling from among all those available from the selected clusters. Obviously, the need for assistance from a knowledgeable biometrician becomes more acute as the complexity of the sampling scheme increases.

7.3.2 Nonresponse

One problem in any sampling system is what is termed nonresponse in the epidemiology of human diseases. These are the individuals that fail to respond to questionnaires or to be included in other sampling exercises. In studies of wildlife these are the individuals in the population that are missed during the sampling procedure, such as the trap-shy animal that is not captured, or the moose that is hidden from view during an aerial survey. These individuals would not constitute a serious problem if their omission simply reduced the number of subjects available for study; however, if they differ from the responders in a characteristic being studied, the resulting bias may

be serious. An example is the situation where older, more experienced, animals in the population avoid capture, so that the resulting sample is biased toward young animals that may have a disease profile very different from the older segment of the population (e.g., Courchamp et al. 2000). The selection bias during evaluating the effects of pesticides on birds, described by Mineau and Peakall (1987) and referred to earlier, is another example of the problem of non-responders. There is no simple solution to the problem of non-responders or other forms of selection bias. The investigator must be conscious of the potential problems and try to ensure that the methods used collect samples that are as representative as possible. When feasible to do so, it may be useful to collect samples by more than one method and compare the results. As noted earlier, if the results are in agreement among samples collected in different ways, one gains confidence in their veracity; if the results are different, one should suspect selection bias and try to find its cause. In some cases, it may only be possible to choose the least flawed from among the methods available.

7.3.3 How many samples?

Hypotheses are tested by the application of statistical techniques and, in general, these techniques are based upon determining if there is greater variation, caused by the effect, between groups than exists within groups. Thus, most tests require an estimate of variation within the groups, as well as of the variation between the groups. In the example of pesticide and robins mentioned earlier in the discussion of hypothesis formulation (Chap. 6), one might count the number of robins in a field before and after spraying, or alternatively count the number of robins in two fields, one of which was sprayed with the pesticide and the other unsprayed. Either method would yield observations that could be discussed but the results would not provide a basis for accepting or rejecting H_0. However, if the number of robins was counted in many sprayed fields, and in a similar number of unsprayed fields (that were comparable in other respects), the amount of variation within each group (sprayed, unsprayed) could be estimated and the variance of the two groups could be compared by some test, such as the F-distribution. This would allow acceptance or rejection of H_0 with a known level of confidence. The important principle is that replication is required within groups if comparisons are to be made among groups. In general, it is preferable to have an equal number of replicates within each group, and some statistical tests require equal sample size for comparison.

The next question to be answered is how many samples or replicates should be collected. This is an important question because collection of more samples than needed is wasteful, and collection of too few samples may lead to results of no practical use. The sample size required should be determined before sampling is begun. The investigator is usually interested in knowing

the minimum sample size that is required. This is determined by the level of precision required, the confidence one wishes to have in the conclusion, and the size of the population from which the sample is drawn. General methods for determining the minimum sample size required for estimating a population mean and a population proportion will be described here; these can be modified for many common situations. The information required for these methods is: (i) a measure of how closely the investigator wants his estimate to approximate the actual value. This is often represented by 'd' and might be a value such as within five units of the true value, (ii) the reliability required of the estimate, i.e., how confident does the investigator want to be of estimating the value closely. Often one wishes to be 95% certain that the estimate will be within ±d of the true value, in which case z_{05} is appropriate, and (iii) the population size which, in wild animals, is usually unknown. The general formula used is derived from: d = z σ/ square root of n, where σ is the standard deviation of the population. Solving for n gives: $n = z^2\sigma^2/d^2$. Notice that in solving this equation, the variance (σ^2) of the population is required, but is usually unknown. Daniel (1983) suggested three methods for obtaining an estimate of σ^2including: (i) the variance of the population estimated from that of a sample collected during a trial collection period, (ii) estimates of the variance available from similar studies on the same subject, and (iii) if the approximate range of variables in the population is known (i.e., if you know the approximate maximum and minimum values expected) and the population is normally distributed, σ is approximately equal to 1/6 the range.

Assume that a parasitologist wished to determine the 'average' number of *Baylisascaris procyonis* worms in adult raccoons. The size of the raccoon population is unknown, but it is very large. A preliminary sample of ten animals had 47 ± 27 parasites. The investigator wishes to have a 95% chance of obtaining an estimated mean within ten of the actual mean number of worms for the population. Using $n = z^2\sigma^2/d_2$ with $z_{.05} = 1.96$, $\sigma = 27$, $d = 10$, then $n = (1.96)^2(27)^2/(10)^2 = 28$. A minimum of 28 raccoons should be examined. The ten raccoons used in the preliminary study could be part of this final sample, providing that no other assumptions about the sample are violated in doing so. If the investigator wished to obtain an estimate within five worms of the population mean, the minimum sample size required would be 112, and if he wished to have a 99% chance ($z_{.01} = 2.58$) of obtaining a mean with the latter precision, a minimum of 195 raccoons should be examined. [Note: because parasites are usually aggregated in a host population (Shaw et al. 1998), calculation of a simple average as I have done here is not useful].

When one wishes to determine an appropriate minimum sample size for estimating a proportion 'p' (e.g., the prevalence or the percent infected) in a population, the method is similar and the appropriate formula is $n = z^2pq/d^2$ where q = 1-p. In this case, there is a need to obtain an estimate of p, which can be done by preliminary sampling or by extrapolation from other similar studies. If it is impossible to estimate p in advance, one can use p = 0.5, (i.e., 50% of the population have the trait), because n is maximal when p = 0.5.

This method will give a sufficiently large sample but it may result in wastage through collection of a larger sample than required. Assume that an investigator wished to estimate the proportion of a deer population that had detectable antibodies to bluetongue virus. He wished to be 95% certain that the estimated p will be within 10% of the true proportion, i.e., if the true proportion is 23%, the estimated value would fall between 13 and 33%. Four of a preliminary sample of 20 deer had antibodies, so he decided to use p = 0.20, and by applying the formula $n = (1.96)^2(0.2)(0.8)/.10^2 = 61.4$. The investigator should collect serum from a minimum of 62 deer (sample size rounded to next entire integer). Tables showing the number of animals required to estimate prevalence under a variety of circumstances are available in DiGiacomo and Koepsell (1986) and Thrusfield (2005) and the values can be calculated using WinEpiscope 2.0 (available at http://www.clive.ed.ac.uk).

The methods above apply to large populations. As a rule of thumb, for large populations the ratio of sample size (n) to population size (N) should be <0.05. If sampling is done from a smaller, finite population, the minimum required sample size for estimating the mean is determined by: $n = z^2\sigma^2/(d^2 + z^2\sigma^2/N)$, and that for estimating the proportion p is determined by: $n = z^2pq/(d^2 + z^2pq/N)$. It is important to remember that these are very general methods and that other techniques for choosing minimal sample size may be more appropriate for a particular investigation. Sample size should be discussed with a statistician prior to any study.

7.4 Is disease present?

A special situation that may confront the disease investigator is the need to determine if a disease is, or is not, present in a population. Requests for this type of information often arise out of conflicts, such as when a wild species is suspected to be a reservoir of infection for domestic animals or humans, or when wild animals are to be translocated from one area to another. In these situations there is often a difference of opinion between those concerned about the wild animals and other interests. The question usually is phrased in a manner such as: "*are deer in this area free of disease X*?". This apparently simple question may have important management and political implications, e.g., whether or not it is safe to import animals from or into an area. While this type of question is often answered by a general statement, such as: "*disease X has never been diagnosed in deer here*", or "*no case of disease X has been seen in a deer since 1999*", these answers are obviously dependent on the visibility of the disease and the diligence with which it has been sought. The saying that **absence of evidence is not the same as evidence of absence** is very relevant in such discussions.

The disease investigator may have to conduct a survey to determine if a disease is present in an area or population. In doing so, one must realize that

while finding a single affected animal proves the existence of the disease in the population, **one can never prove with absolute certainty that a disease does not exist in a population unless every single individual in the population can be examined using a technique that is infallible (sensitivity = 100%)**. Since no technique is infallible and, in most cases it is impossible to examine all of the animals, it is important to explain **in advance** that an absolute answer is impossible. The investigator must communicate that, on the basis of testing, one can only be confident to a defined level of certainty that the prevalence of the disease is not greater than a specified level in the population. This is a concept that is difficult to explain to individuals who want a Yes/No answer.

Methods to determine minimum sample size required to be reasonably certain of detecting at least one diseased individual in a population of given size, among which the disease is occurring with a specified prevalence, have been described by several authors, (Ossiander and Wedemeyer 1973; DiGiacomo and Koepsell 1986; Thrusfield 2005) and sample size can be calculated using WinEpiscope 2.0.

As an example of making such a calculation, assume that we are asked to determine if tuberculosis is present in an elk population containing approximately 1,500 animals. Some preliminary results indicate that if tuberculosis is present it is unlikely to occur at a prevalence greater than 1%. Using WinEpiscope 2.0, we can calculate that with a sample of 271 elk (or 18% of the population), and if the prevalence is 1%, the probability of diagnosing at least one animal as positive is 95%. If we examine a sample of this size and do not find an infected animal, we cannot say that the population is free of tuberculosis, but we can report that we can be 95% confident that the prevalence is not greater than 1%.

Tables in Thrusfield (2005) and WinEpiscope 2.0 also can be used to find the upper 95% confidence limit to the number of diseased animals in a population, from which a sample of a particular size was tested and found to be negative.

In the early stages of sampling, or when asked for an immediate reaction to a sampling plan, a simple rule of thumb may be useful. The "***rule of three***", is based on the observation that "*if none of n patients shows the event about which we are concerned, we can be 95% confident that the chance of the event is at most 3 in n (i.e., 3/n)*" (Hanley and Lippmann-Hand 1983). In other words, we can be 95% confident that the prevalence is not greater than 3 divided by the number examined. For example, assume that we are asked to comment on whether examining a sample of 32 bison without finding any evidence of brucellosis means that the population is free of the disease (this sort of question does in fact arise!). Applying the rule of three, the prevalence is not greater than 3/32 or .09, so we could advise that, based on this sample, we can be 95% confident that the prevalence of brucellosis in the population from which the sample was collected was not greater than 9%, assuming that the test had a sensitivity of 100%, (which is unrealistic). Values obtained

using the rule of three approximate values calculated by more exact methods when n is larger than 30; with less than 30, the rule tends to overestimate the risk slightly (Hanley and Lippmann-Hand 1983). The rule of three can also be used to determine an appropriate sample size, given a specified level of confidence and a suspected prevalence of disease, by applying the formula n=3/prevalence. If we used the rule of three to calculate the minimum sample size (n) required to be 95% confident of detecting at least one diseased individual from the elk herd described earlier, n = 3/0.01 = 300. Hanley and Lippmann-Hand (1983) suggested that a "*rule of 4.6*" and a "*rule of 6.9*" can be used for a 99% and 99.9% confidence interval, respectively. This rule of thumb is useful in the early planning of a survey, when it is nice to know whether one needs to collects tens, hundreds or thousands of samples. It also is useful when someone suggests that examining five or ten deer should be adequate to establish if a disease is present or absent. The sensitivity of tests is always < 100% and this will reduce the ability to detect disease (Greiner and Gardner 2000; Joly and Messier 2005). Formulae for making corrections are available but are complex (Thrusfield 2005).

While systems for choosing sample size may be mathematically correct, the biological implications must be considered. Diefenbach et al. (2004) modeled the effectiveness of tests conducted on hunter-killed deer for detecting chronic wasting disease (CWD) in Pennsylvania, a state with an estimated population of 1 million deer ≥ 1 year of age. They suggested that a sample of about 500 deer would have a 99% chance of detecting at least one deer with CWD if: (i) the prevalence was ≥ 1.0%, (ii) the disease was uniformly distributed, and (iii) all deer had an equal chance of being sampled. However, at 1.0% prevalence there would be approximately 10,000 deer with CWD, and even at 0.1% prevalence there would be 1,000 affected deer in the state. Certainly a manager would like to detect the disease before this many deer were affected. In jurisdictions where CWD occurs, the disease usually is not uniformly distributed. Diefenbach et al. (2004) modeled a situation in which deer were sampled from all 22 wildlife management units (WMU) in Pennsylvania, but CWD was present in only one WMU, at a prevalence of either 1% or 0.1%. The number of deer tested from each WMU was proportional to the percent of the total state-wide deer population present in that WMU. The test was assumed to have a sensitivity of 100%. Under these conditions, > 25,000 deer would have to be tested statewide to have a > 50% probability of detecting a deer with CWD. All available information on the past history of the area, the biology of the species, and a good deal of common sense must be used, together with any survey results, and targeted surveillance, such as testing any deer with neurological disease in the case of CWD, to answer the question as to whether a disease is present or not.

The methods that will be used to test H_0 should be chosen prior to sampling, in the same way that other parts of the methodology are chosen. It is wise to examine data collected during a trial sampling period to ensure that

the data are compatible with the method chosen. Each statistical test requires certain assumptions about the data, and the type of data collected during disease studies seldom satisfies these assumptions completely. The critical assumption for most statistical techniques is that the sampling is done in a random manner; if this is not true, the results are unlikely to be valid. Other important assumptions relate to the normality of the data and the homogeneity of within-group variation. In examining data from a preliminary trial, one should identify the assumptions required by the proposed method and then determine which, if any, of these have been violated. Assistance from a statistician will probably be required to decide the consequences of the violations on the validity of the test (i.e., is it broken, or just sprained badly?), and for transformations of the data that may reduce the violation. In some cases, the violations may be such that non-parametric methods are required. The method chosen should be as conservative (low probability of making a type 1 error), powerful (low probability of making a type 2 error), and robust (not seriously affected by the vagaries of environmental data) as possible (Green 1979). Consideration should be given to using methods other than standard statistical tests, as proposed by Johnson (1999).

7.5 Summary

- The most important consideration during any type of sampling is to ensure that the samples are representative of the population from which they are drawn.
- Samples may be non-representative because of random error or because of bias.
- Of the three basic types of bias (selection, measurement, confounding), selection bias is most common in samples collected from wild animal populations.
- It may be impossible to totally prevent bias, or to measure its extent, but it is usually possible to determine its direction and to use this information in interpreting results.
- When collecting samples from animals, always consult in advance with the person who will do the analysis regarding the best methods for collection and preservation of specimens.
- Unlabelled specimens or specimens that lose their label are of no value.
- No analytical test should be used without a full understanding of its suitability for the wild species being studied. Tests developed for domestic animals or humans often are applied to wild animals without ensuring that they are suitable.
- Sampling design should always be developed in consultation with a knowledgeable biometrician.

- Extraneous variability may be reduced by restriction in choosing specimens for sampling.
- Specialized methods including stratified sampling, cluster sampling, systematic sampling and multistage sampling are often more appropriate than simple random sampling.
- Replication is required within groups if comparisons are to be made between groups.
- Mathematical techniques are available for determining appropriate sample size. Sample size should always be determined prior to starting data collection.

8 Investigation of disease outbreaks and chronic or inapparent disease

> "*I cannot, of course, write too vividly of the interest which the study of epidemics has brought me personally, quite apart from the conviction that it must inevitably lead in some small way to an addition to our knowledge of epidemic diseases*"
>
> (Pickles 1939)

Disease in populations occurs across a spectrum from sudden explosive outbreaks in which there is obvious morbidity or mortality, to very chronic and protracted conditions in which it may be difficult to detect sick animals or any other evidence of the disease. Disease in wild animals is often likened to an iceberg with the great bulk of the problem hidden from view. In this analogy, the exposed tip is often the disease outbreak, while chronic disease is that portion hidden most deeply underwater. Each of these extremes presents some peculiar difficulties for the disease investigator. Outbreaks are usually short-lived, transitory phenomena that demand immediate attention and that often excite public and media attention. This may result in considerable pressure to find an answer as to the cause. There may also be an expectation that management should be instituted which, in many cases, may not be practical or justified. In contrast, the problem with insidious diseases is often one of identifying affected animals and of assessing the effect of the disease. Chronic diseases may have important effects on populations but, because of their covert nature, it may be difficult to convince administrators or the public, of their occurrence and of the need for their investigation or management. This chapter will deal with techniques particularly suited for the investigation of these dissimilar types of disease.

8.1 Investigation of outbreaks

Most disease outbreaks in wild animals are ephemeral events that began long before they were recognized or reported and that may already be in decline by the time one has the opportunity for investigation. In some situations this may be fortuitous, in that the problem disappears before one is forced to admit that management is impractical! However, it also can be very frustrating when one is attempting to study and understand the disease. **Because of the**

transitory nature of outbreaks, one should treat each reported occurrence as a matter of some urgency and collect as much information as possible while the opportunity is available.

Investigation of an outbreak, in my experience, usually begins with a telephone report of sick or dead animals. It is important to collect information from the reporter to assess what type of investigation is possible or justified. In Chap. 9 I will discuss the use of a general disease-occurrence record sheet. This type of record sheet is very useful for collecting information in the early stages of an outbreak. The information collected should include the name, address, and telephone number of the reporter, the precise location of the outbreak (including methods of access to the site and names of any landowners involved), the number and species of animals involved (i.e., sick or dead), clinical signs or unusual features observed in these animals, number and species of animals present in the area but apparently not involved, timing of the outbreak (has it been going on for hours, days, or weeks?), unusual events or circumstances thought to be associated with the outbreak, such as changes in weather, agricultural practices, or management activities, and the names of persons from other agencies involved (to reduce redundancy and allow cooperation). The possibility of collection and submission of specimens to the laboratory by the reporter should be discussed, and the need for special equipment, such as boats or all-terrain vehicles to reach the site and to collect specimens, should be established at this time.

This information forms the basis for deciding if a field investigation is necessary and it may also indicate the extent of study that is justified. In some instances, all that is required is the identification of the etiologic agent. Submission of a sample of affected animals to the laboratory may be sufficient in such cases. In other situations, one may wish to know more about the disease such as WHERE it is occurring and how large an area is involved, WHAT the circumstances are that resulted in the occurrence and WHY the disease is occurring. **Whenever possible an investigator should visit the site of the outbreak** because there is no substitute for direct observation. As an example, in 2005 a conservation officer submitted a single dead deer mouse that had been found along with many other dead mice near a granary in a farming area of western Saskatchewan. We diagnosed tularemia (*Francisella tularensis holarctica* infection) in the mouse by necropsy and bacteriologic examination, and that could have been the end of the story. However, because I was able to do a follow-up field investigation, we discovered that there had been a massive irruption of deer mice over a >22,000-km^2 area, with extensive mortality at least partially as a result of tularemia. In this epizootic, as in many others, most of the mortality occurred prior to the disease being detected, and what appears to be the first recorded epizootic of tularemia in deer mice would have gone unnoticed except for the submission of a single mouse!

The first visit to a disease site should be viewed as a reconnaissance to collect data that will be used to form a working hypothesis about the disease. However, because of the transient nature of many outbreaks, the investigator must go

equipped to collect a variety of specimens and information on this first visit, as there may be no tomorrow! The amount and type of equipment that can be taken into the field is determined by the disease suspected, the method of transport, and the ability to return perishable specimens to the laboratory. It usually is impossible to foretell the type of samples that may be required or available, so both the equipment taken and the investigator must be adaptable. **Where possible one should strive to take replicate samples of anything that might potentially be of use.** It is much more satisfying to have the luxury of discarding extraneous samples than to wish, at some later date, for unattainable, uncollected samples.

8.1.1 Basic equipment

The choice of equipment taken into the field is highly personal. The following list includes equipment that has served me in a variety of circumstances. The list can be added to as required. All of this equipment fits into a wooden field case and a plastic cooler and this kit has survived travel in various types of aircraft, boats, and all-terrain vehicles.

Basic equipment for investigation of outbreaks includes:

Necropsy: knife, sharpening steel, scissors, forceps, scalpel and blades, small (hack) saw, disposable rubber gloves, rubber apron, small butane torch or alcohol lamp, matches, sterile syringes and needles, sterile swabs with transport media.

Measuring: tape measure, thermometer, spring scale.

Recording: field note book, record sheets, pencils, permanent markers, adhesive tape for labels, camera with macro/zoom lens, extra film or storage system for digital images, GPS unit for identifying locations, batteries.

Containers: plastic bags of various sizes, including garbage bags and sterile rip-top bags, sterile plastic vials, tubes for clotted and unclotted blood, aluminum foil for wrapping specimens, canvas sack (mailbag type, for everything from live ducks to moose heads), glass slides for blood smears.

Preservatives: 10% buffered formalin (if weight or volume are a concern, I sometimes take concentrated formaldehyde and preweighed dry buffer and make up formalin on site), 70% alcohol (also used for flaming instruments), 4-l plastic jug filled with water and frozen for cooler.

General: binoculars, maps of the area, coveralls, disinfectant soap, disinfectant for boots, paper towels. Because of the risk of zoonotic disease, as in the case of hantavirus and small rodents, additional personal protective equipment (e.g., Mills et al. 1995) should be available and used.

8.1.2 Basic procedures

Although the methods and procedures used will vary among different outbreaks, certain basic steps are common to most investigations, including: (i) definition of the problem (What?), (ii) collection of population and environmental data

(Who, When, Where?), (iii) formulation of a hypothesis to explain the outbreak (Why?), (iv) testing the hypothesis, and (v) recommendation of appropriate action.

The first step is to decide if a significant outbreak is actually occurring. Usually this is not a problem; however, we have seen instances in which an outbreak of disease was suspected when several deer, killed by gunshot and collision with automobiles, were found in an area over a short period of time. Conversely, the first recognized outbreak of epizootic necrotizing enteritis, a serious infectious disease in wild geese, was overlooked for several months because it was assumed that the birds seen dead were part of 'normal' crippling loss, i.e., birds wounded by hunters in the area (Wobeser and Rainnie 1987).

8.1.3 Identifying the cause

The next step is to identify the cause of the disease. The most important method for doing this is through collection and necropsy of a sample of animals suspected to have the disease. Whenever possible, a sample of animals should be necropsied in the field as soon as it is practical to do so during an investigation. The purpose of these field necropsies is to establish a tentative hypothesis about the nature of the disease, so that **appropriate** specimens can be collected for detailed examination in the laboratory. It is crucial that some animals be examined by a trained pathologist supported by full ancillary laboratories (microbiology, parasitology, toxicology, etc.) to ensure that abnormalities are properly identified and interpreted. In some cases, it is possible to make a definitive diagnosis in the field and, in most situations it is at least possible to identify the general type of disease present and to rule out some potential causes. For instance, if I were called to a die-off of waterfowl in midsummer in Saskatchewan, I would suspect botulism as the probable cause and would collect serum samples from a sample of sick birds for toxicity testing. However, I would necropsy some birds, and if I found lesions during the field necropsies, I would collect additional specimens for laboratory examination for parasitic, bacterial and viral pathogens, as well as for other toxicants.

A further step is selection of specimens for laboratory examination. Whenever possible, a sample should be selected that is both representative of the problem and suitable for examination. This may be difficult and one may have little choice if only a few affected animals are available. If many specimens are available, as in die-offs of gregarious or colonial birds, stratified random sampling should be used to ensure that all species and age groups are represented (see Chap. 7). The sample should consist largely of sick and recently dead individuals, as these are much more likely to yield useful information than are decomposed carcasses. However, if only decomposed carcasses are available, they are much better than nothing, but consult with the pathologist who will have to examine them! Minimum sample size is discussed in Chap. 7 and the principles cited there should be employed when possible. Often sample

size is limited by the number of specimens available or by the number that the laboratory can or will examine. In situations where more specimens are available than the laboratory can handle, we usually choose a sample for initial examination and preserve additional animals by refrigeration or freezing, pending the results of the initial laboratory examination. In this way additional specimens are available if required later. Often, as in the case of the tularemia outbreak described earlier, the causative agent is identified during the initial laboratory examination and this may represent the end of the investigation. If further information is needed, identification of the cause is only the first step. Even if the cause can not be determined, a working definition of the disease should be drawn up for identification of further cases. This definition should be as specific as possible and include the epizootiologic, clinical and pathologic features that have been observed to this point in the investigation.

8.1.4 Collection of epizootiological data

The collection of population and environmental information to answer the Who, When, and Where questions about the disease is an important part of the investigation. The objective is to identify those factors that are associated with the occurrence of the disease and that do not occur when and where the disease is absent. Although I have presented the collection of information as a step-wise progression, in reality the collection of this information begins at the start of the study. These subjects have been discussed in detail in earlier chapters and will only be reviewed briefly here. A major consideration in the investigation of any disease outbreak should be determining the distribution of events in space and time. One should be particularly vigilant for clustering of events that may provide valuable leads. Mapping of disease events, and particularly of all identified cases, should be a standard part of the investigation and may provide important leads to the source or nature of the causative agent or to the way in which animals are being exposed (GPS technology has made mapping of disease events an easy task). Events also should be plotted against a time scale. A problem in the investigation of disease outbreaks in wildlife is that the initial stages of the outbreak are often long past before the investigation begins. However, it may be possible to reconstruct events retrospectively and to identify the approximate time of onset by interviewing people who are knowledgeable about the area, by determining the relative proportion of the carcasses that are fresh, as compared to putrefied or desiccated, or by using other indicators of time. We were able to estimate the timing of a disease outbreak that occurred among geese in the arctic by comparing the plumage of goslings found dead to the normal phenology of feather growth (Wobeser et al. 1982) and we were able to estimate the timing of an outbreak of necrotizing enteritis in geese by the position of carcasses in and on the ice of a lake in relation to the known date of freeze-up of the lake (Wobeser and Rainnie 1987).

A method commonly used in epizootiology is to plot the occurrence of disease events, such as new cases or deaths, graphically in the form of an epizootic curve that illustrates the rate of change in occurrence of the disease over time. The nature of the curve varies with the causative agent, the method of exposure or transmission, and the number of susceptible animals exposed. Some types of disease result in a well-defined pattern that may be useful in identification of cause. For example, a single sharply defined peak or cluster of disease events suggests that the animals were exposed to a **point** or **common source** of the causative factor over a short period of time. We observed an extreme example of this when many California gulls died within a 2-h period in a field. We subsequently found that the field had just been sprayed with the pesticide carbofuran. Continuous exposure to a source of the causative factor may result in a protracted or drawn out epizootic curve. This is the type of pattern that might be expected among ducks utilizing a marsh heavily contaminated with lead shot. Intermittent exposure to a common source may result in a series of peaks or clusters. Infectious diseases that are transmitted either directly or indirectly from one host to other susceptible animals often produce a so-called progressive or propagative epizootic curve. The same is true in many outbreaks of botulism in ducks, in which the number of new cases increases as a result of the carcass-maggot cycle. If a new disease is introduced into a population containing many susceptible animals there will be a gradual increase in the number of cases and then, as the epizootic continues and the supply of susceptible animals declines, the number of new cases tapers off and finally stops. The rate of increase in the number of cases during the early stage of the outbreak depends on the rate of transmission, the length of the incubation period, and the number and location of the susceptible hosts. Some infectious diseases may produce a wave-like pattern, with the period between waves or clusters of cases corresponding to the incubation period of the disease.

During outbreaks, it usually is easier to estimate the number of affected animals than it is to determine the population size; however, both the number affected (the numerator) and the population size (denominator) are required for calculation of the various rates, such as prevalence and mortality, that indicate the severity of the outbreak. The relative rate of occurrence in various species, and within different sex and age groups, may provide important clues to the nature of the disease. The accuracy of estimates of the number of individuals affected depends on the method used in searching for sick or dead animals, the frequency of searches, and the rate of recovery and/or removal of diseased animals by predators and scavengers. Sometimes the area involved is so extensive that it is impossible to search it entirely, so that some form of sampling is necessary. Many of the techniques discussed in Chap. 4 and those illustrated in Figs. 7.1 and 7.2 are appropriate for this purpose.

An example of how this type of sampling can be modified to fit a circumstance was described in Fig. 7.2. We used the method during an outbreak of

avian cholera among ducks on a large lake. Because of the size of the lake it was impossible to search the entire shore for sick and dead birds, and the length of shoreline was unknown. We decided to systematically search 10% of the shore for carcasses, and we wanted our sample to be representative of the entire shoreline. We did this by proceeding around the shoreline, measuring the distance as we went, using a 50-m cable dragged behind an all-terrain vehicle. Prior to starting, we randomly chose one 50-m segment to be searched within each 500 m of shoreline. Using this method, two people were able to search 10% of the shoreline intensively in a day. Because we traveled the entire shoreline we made qualitative observations about the distribution of sick and dead birds and located dense accumulations of dead birds in association with two inflow streams from which we subsequently isolated *Pasteurella multocida*. This method has features of cluster sampling (see Chap. 7) with each 500-m stretch of shoreline being a cluster or subgroup, from within which a 50-m sampling area was randomly chosen (a count of dead animals of this type is a measure of the prevalence of carcasses at the time of the search and it is not a measure of cumulative mortality, unless all the animals that died are still present, Table 4.2).

Use of simple techniques, such as mark-recapture (e.g., marking a known number of carcasses prior to a search for carcasses) or observing the rate of removal of marked carcasses by scavengers, also may be done to measure the efficiency of the search procedure and to give an estimate of the proportion of carcasses that are removed by natural means each day. We used mark-recapture during a botulism outbreak among ducks and found that only about one-third of marked carcasses were recovered during routine cleanup operations. There were distinct differences in the recovery rate of different species of birds, perhaps based on the size and visibility of the carcasses. For example, about 53% of marked mallard carcasses were recovered, while only 25% of the carcasses of smaller species, such as American coots and blue-winged teal, were recovered (Cliplef and Wobeser 1993). Mark-recapture also can be used to estimate the total number of animals affected at any time in the area. For example, during a botulism outbreak, we marked 43 bird carcasses in one part of a marsh. During the subsequent cleanup done by other workers, 43 carcasses were collected, of which 12 were marked. Thus, only about 28% of the carcasses present were collected and the estimated number of carcasses present at the time of the carcass collection = $43 \times 43/12 = 154$.

Methods for estimation of the total population present in an area have been discussed in Chap. 4, and their use during an outbreak will depend on the resources available. Even in situations where it is not possible to collect detailed quantitative information, it is important to record the relative abundance of various species, and the sex and age composition of both the affected and unaffected portions of the population. Many diseases have a restricted host range, and differences in factors such as food habits or behavior may be important in restricting disease to a certain species or to some group within

a species. Information on the relative rate of occurrence may provide important insights into the nature of the disease. However, despite good intentions, the data that may be available often are incomplete. For instance, during an outbreak of classical swine fever in wild boar, only 77 boars were found dead but 5.5 months after the outbreak began, only 87 of the 465 susceptible animals present in the area prior to the outbreak remained alive, i.e., 301 animals could not be accounted for (Hone et al. 1992).

Collection of information on environmental variables was discussed in Chap. 5. In the investigation of an outbreak, one is interested in events, and particularly changes that occurred immediately prior to the outbreak and that may continue to occur during the outbreak. Among the variables that should always be considered are weather, changes in the population of animals or recent movements (particularly in migratory species), and agricultural and other human activities in the area. Some of this information can be obtained retrospectively through interviews with people in the area and from weather records, but it is important to measure variables on the site because of the importance of micro-environmental factors. It is useful to incorporate environmental data on maps and graphs of the spatial and temporal distribution of disease events. A simple procedure such as combining wind direction information with that on disease occurrence may explain the occurrence of a disease in an area in some situations.

A single visit to an outbreak site provides a snapshot of the situation. Further monitoring is required to determine the temporal characteristics of an outbreak. When an outbreak is followed over time, it is important to examine additional specimens on a regular basis to ensure that the disease has not changed. Often more than one disease may be occurring in a single outbreak and the nature and cause of mortality may change. One should never assume that animals dying near the end of an outbreak are succumbing to the same disease as those that died at the beginning, or that all of the species found dead during an outbreak died of the same disease. I once investigated a die-off of ducks that appeared to have begun as a result of cyanobacterial (blue-green algal) poisoning. The carcasses of the poisoned birds became substrate for proliferation of *Clostridium botulinum* and mortality, now caused by botulism, continued long after the algae had disappeared. In this instance, mortality continued at the site over an extended period of time but the cause and nature of the disease had changed completely. We have seen a situation in which a number of double-crested cormorants died on an island. The cause of death of these birds was not determined at the time but the possibility of botulism was eliminated by extensive testing; however, as the outbreak in cormorants declined, paralyzed ducks and Canada goose goslings were found on the island. These birds had botulinum toxin in their serum and we assume that decomposing cormorant carcasses provided a source of toxin-laden maggots for the waterfowl. The primary disease in the cormorants was subsequently found to be Newcastle disease, but we were unable to find evidence of that disease in the waterfowl.

8.1.5 Application of analytical techniques

In the 'fire-fighting' atmosphere prevalent when a major outbreak occurs, relatively little thought is usually given to application of the analytical techniques described in earlier chapters. Emphasis is placed on obtaining a diagnosis and instituting management procedures, and the information that is collected is descriptive in nature. The basis for all of the analytical techniques is comparison between the affected and non-affected portions of the population and these techniques can provide information not obtainable by other methods. A requirement for these techniques is that the disease must be defined in sufficient detail so that **cases** can be distinguished from **non-cases**. Two methods, the cross-sectional or prevalence survey and the case-control method (described in Chap. 6) are most suitable for use in the investigation of outbreaks. Some aspects of the descriptive process, such as comparing the species, sex and age composition of the diseased individuals to that of the population at large, resemble a cross-sectional study. However, if one wishes to make more direct comparisons, such as the concentration of a toxin or presence of antibody to an infectious agent in affected and non-affected individuals, some form of sampling is necessary. If a cross-sectional survey is used, a sample can be collected from the population and comparisons made between the prevalence of various factors in affected and non-affected individuals from within this sample. The disadvantage of this method is that a large sample may be required to obtain sufficient cases for comparison. The case-control method has the obvious advantage that cases are identified first and then appropriate controls can be selected from the population by various forms of matching. This method has received relatively little attention in the investigation of disease outbreaks in wild animals, but has proven useful in the early stages of investigation of epidemic diseases in man, including Legionnaire's disease (Fraser et al. 1977) and toxic shock syndrome (Langmuir 1982).

The purpose of investigation of an outbreak is to allow formulation of a hypothesis that will explain why the outbreak occurred. Once a hypothesis is available, it can be tested in various ways, such as through collection of further specimens that relate to the hypothesis, use of the analytic techniques described above, or by manipulation of factors thought to be important in the epizootiology of the disease. The other function of the investigation is to assess the significance of the outbreak and to determine methods that might be used either to manage the present outbreak or to prevent future outbreaks.

8.2 Investigation of chronic or inapparent disease

The disease conditions discussed in the first half of this chapter become visible because many individuals become sick or die over a short time period. The accumulation of diseased individuals may overload the normal predation/

scavenging system, so that the surplus diseased animals are visible to the human observer. At the opposite extreme are those diseases that are gradual in onset, have a prolonged course and that may never result in morbidity or mortality at a level sufficient to overload the scavenging system. In the epidemiology of human diseases, the term chronic disease often is used to indicate non-infectious conditions, although this is a misnomer. Here, chronic will be used to refer to disease of long-duration, regardless of etiology. Chronicity may result from prolongation of either or both of the period between initiation of the disease process and the onset of clinical disease (sometimes called latency), and/or the period between the onset of clinical disease and termination. In some diseases, such as degenerative joint disease, both phases may be prolonged, whereas in other diseases such as malignant catarrhal fever in deer there is a prolonged period between initiation (infection) and the onset of clinical disease, but the clinical course may be short.

The investigator usually is interested in the same types of information required in any disease, i.e., rate of occurrence, cause, and effect on the population, but chronic diseases present a special problem because of their covert nature and the necessity of following individual animals for an extended period of time in order to understand the disease. The first major problem is often to detect if a chronic disease is present in the population. This may be difficult if the disease is clinically silent for long periods and if, as is usually the case, animals that do become ill are removed by predators and scavengers. With chronic disease "*typical warning signs that disease is modifying host population dynamics, specifically changes in population size and age structure or sudden mass mortalities, may not be present despite large effects on population growth rate*" (Jolles et al. 2005).

8.2.1 Using laboratory records

Diagnostic laboratories are a particularly valuable surveillance mechanism for detecting chronic disease in a population, because of the continual examination of animals and recording of the results done in these facilities. Individual cases may be encountered infrequently, and the accumulation of sufficient cases for meaningful analysis may require many years but, if the research is done as part of the routine activities of the laboratory, it may involve little or no added cost. A study in Sweden serves as an example of the usefulness of records from diagnostic laboratories for detecting chronic or rare diseases. Borg and Nilsson (1985) documented the occurrence of malignant ethmoid tumors in moose and roe deer in Sweden based on animals necropsied between 1947 and 1982. During this 35-year period they accumulated a series of 35 moose and four roe deer with this tumor. Based on this sample, they were able to describe the geographic range of the condition, the sex-specific prevalence (all cases in moose were in females), and to advance a testable hypothesis about its cause. The prevalence of the condition among animals

submitted to the laboratory was only about 1% in moose and 0.1% in roe deer, and the prevalence in the general population was thought to have been even lower. Using the 'rule of three' to calculate the minimum sample size required to detect a diseased individual, one would have to examine a sample of approximately 600 moose to be 95% confident of finding a single affected animal, assuming that the prevalence in the population was 0.5% , i.e., half that in the necropsied animals. It is highly unlikely that anyone would finance a cross-sectional research survey large enough to detect 35 affected moose!

Careful record keeping is essential for detection and long-term study of chronic diseases in a population. This was demonstrated in pioneering work on the effects of chlorinated hydrocarbon pesticides on birds of prey. The relationship between environmental accumulation of these compounds and changes in eggshell thickness, with resulting declines in breeding success and populations of raptors (Ratcliffe 1970; Newton 1998), was evident only because records of the breeding biology of these birds over an extended period of time were available.

8.2.2 Sampling to detect disease

Another method of studying chronic disease in wild animals is through repeated cross-sectional surveys. For example, annual samples of hunter-killed deer were used to monitor the occurrence and prevalence of renal urolithiasis (calculi or stones in the urinary tract) in a herd of white-tailed deer (Woolf et al. 1976). Such surveys provide only snapshot views of the disease but a series of such views may allow reconstruction of the pattern of disease development in the population. A problem with this type of study for the study of chronic diseases is that cross-sectional surveys detect both recently acquired and long-standing cases, and usually do not provide a measure of the incidence of the disease, i.e., the rate at which new cases are occurring.

It may be important in some situations, such as in disease-management programs, to know whether or not the incidence of a disease is changing. As an example, assume that a control program was instituted in March of 2000 to reduce fluoride emissions from a chemical plant and the resulting occurrence of fluorosis in deer in the area (most of the fluoride reaches deer via dust on vegetation rather than from the soil). The only logistically feasible method available for monitoring occurrence of fluorosis was an annual cross-sectional survey of deer killed by hunters during November of each year. Permanent teeth from the hunter-killed deer were examined for pitting and excessive wear typical of lesions caused by exposure to large amounts of fluoride. Tooth lesions occur only if the animal was exposed to excess fluoride while the teeth were developing but, once present, the changes are permanent. Prior to the beginning of control measures, one would expect the prevalence and severity of lesions in permanent teeth to be similar in adult deer of all ages. Even if the control program is effective, several years will pass before the

overall prevalence of the disease in the population will change markedly. However, one can estimate the incidence by looking at the age-specific prevalence of fluorosis each year. The most useful cohort to examine will be those deer that are approximately 2.5 years old during the hunting season, since this is the first group in which all permanent teeth have erupted. In the 3 years prior to beginning control of emissions, the prevalence in 2.5-year-old deer averaged 16 ± 3%. In November 2000, 7 months after controls began, one would not expect the prevalence in 2.5-year-old deer to have decreased, because these deer were exposed to fluoride throughout most of the time that their teeth were developing. In November 2001, there would probably be lesions in some teeth of deer in the 2.5-year-old cohort, but the teeth that are the last to develop and erupt may be free of lesions. In November 2002, deer in the 2.5-year-old cohort should be largely free of tooth lesions (if the control measure is effective) but the prevalence of lesions will be unchanged in older deer. With time, the proportion of deer born prior to control will decrease and the overall prevalence will fall.

The incidence rate for a specified period of time, which is a measure of the risk of acquiring a disease, can be estimated from repeated measurements of prevalence in a cohort of young animals born into the population and initially free of infection (Schwabe et al. 1977). Comparing the incidence rate before and after the management provides a measure of the effectiveness of the management. To illustrate, assume that we want to reduce the rate of infection with the liver fluke *Fascioloides magna* in a population of elk and that we will do this by reducing the number of snails that act as an intermediate host for the parasite. We will use the presence of fluke eggs in elk feces to distinguish between infected and uninfected animals. Prior to beginning management we find that the prevalence of infected animals (those with fluke eggs in their feces) in a group of 12-month-old elk is 18%. We are able to measure the prevalence in animals of the same cohort 6 months later when they are 18 months old, and find that the prevalence is now 24%. The incidence rate (IR) for 1 year can be calculated by the formula: $IR = 1 - x^{12/y}$ where x equals the proportion of the cohort that was free of disease on the first examination (0.82) that was still negative at the second examination (0.76) (Schwabe et al. 1977). To calculate a yearly IR, y equals the number of months between surveys. In this case, $IR = 1 - (0.76/0.82)^{12/6}$ or $1 - (0.927)^2 = 1 - 0.86 = 0.24$ or 24%. After management has been instituted, another pairwise comparison can be made. When the new cohort is measured at 1 year of age, the prevalence is 16%, which is not very encouraging, but when they are measured again at 18 months, the prevalence is 19%. Now the IR is $1 - (0.81/0.84)^{12/6} = 1 - (0.964)^2 = 1 - 0.93 = 0.07$ or 7%, suggesting that the risk of acquiring liver flukes has declined substantially coincident with management.

Indirect tests, such as for serology, may be the most efficient way to monitor the prevalence of chronic infectious disease in a population but one must be concerned about the specificity and sensitivity of the test, because errors in disease testing (misclassification of individuals as being diseased or not diseased)

can lead to underestimation of the effects of disease (Joly and Messier 2005). Investigators should be concerned about how long animals remain seropositive after exposure, and particularly cognizant that immune function may be impaired in chronically diseased or debilitated animals. For example, some ungulates with disseminated tuberculosis are negative when tested by the standard tuberculin test.

Many techniques have been developed for study of the relationship among various factors in chronic disease in human populations. Of these, the long-term, prospective cohort study has been most productive. A study of the risk factors associated with cardiovascular disease in the population of Framingham, Massachusetts, is the most famous of these and serves as a model for most other studies. That study involved following several thousand 30 to 59-year-old persons over a 20-year period, with biennial examination of each person and continuous monitoring of their exposure to a large number of variables. At the completion of the initial 20-year period, the study was continued and expanded because of its productivity (Dawber 1980). No comparable cohort study has been attempted in wild species to my knowledge, and to do so would be extremely difficult because of the problem of following individuals over an extended period. This technique could be applied to sedentary species, or to birds with a high degree of nest site fidelity, in which it is possible to recapture the same individual periodically and thus monitor the development of a disease in relation to other variables. Spieker and Yuill (1976) used a form of this type of study to investigate *Herpesvirus sylvilagus* infections of cottontail rabbits. They live-trapped rabbits repeatedly to study the relationship between the number of fleas on these animals and the occurrence of antibody to the virus. No correlation was found between the number of fleas and antibody titres but disease transmission during winter was demonstrated by observation of seroconversion in individual animals. A study of *Mycobacterium bovis* infection in European badgers (Chesseman et al. 1988) is a good example of a form of cohort study. Groups of animals were followed by periodic sampling for several years to determine the developmental sequence of tuberculosis in both individual and groups of badgers.

The cohort approach also can be used to study chronic disease through the longitudinal study of a group of individuals known to have been exposed to a specific risk factor. This method has been used in human medicine to study the long-term effects of radiation among Japanese who survived atomic bombing. It would be equally applicable for studying the effects of radiation from the Chernobyl disaster on long-lived animals, such as Scandinavian reindeer and moose. The study of survival of mallards exposed to lead shot by Bellrose (1959) is one of the few examples available of the use of this technique in wild animals.

Another technique that is used widely in the study of chronic disease in human populations is the case:control method. This technique depends on the identification of affected individuals (the cases) and comparison of characteristics of these individuals to those of selected controls that do not have

the disease. It is particularly appropriate for uncommonly detected or rare diseases, because it can be applied as individual cases are discovered. In humans, the backward-looking or retrospective form of this technique is very useful for the study of chronic diseases, because it collapses the time period between probable exposure and the recognition of clinical disease through the examination of the medical records of the individual, or by interviewing the individual. Thus, one might identify individuals with and without a disease and then determine, by interview or by consulting medical records, if they have been exposed to a specific risk factor in the past. The lack of recorded history and inability to interview the subjects means that this technique is generally not available to the investigator studying wild animals. However, antibody titres and residues of certain persistent environmental pollutants in tissues, e.g., mercury in pelage and plumage and lead in bone, are a form of historical record of the experience of the animal that could be used in this way. Similarly, one might be able to use a retrospective approach if a risk factor is known to have occurred in an area at some time in the past. This approach has been used to compare barn swallows exposed to radiation from Chernobyl with non-exposed controls (Møller et al. 2005). A form of case:control study was used to determine the effects of wing tags on gulls (Kinkel 1989). Cases were marked with wing tags, while control gulls were marked with colored leg bands. By following the birds over several years, it was found that fewer wing-tagged birds returned to the colony, those that did return arrived later and their eggs hatched later, and pair bonds of tagged birds were broken more often, compared to the controls. Thus, a difference in mortality rate and subtle effects on reproduction and behavior associated with a risk factor (wing tag), that was producing chronic disease, were demonstrated. This study provides an excellent example of the need for long-term research to discover the lasting effects of a chronically active risk factor.

The more common method of using the case:control technique is in a non-directional manner in which one observes the disease and the factor under consideration simultaneously. For example, if we were interested in a relationship between a chronic skin condition and the thyroid gland, we might compare the thyroids of animals with the skin condition to those of appropriate controls that had normal skin. This approach might provide evidence of an association between the two factors but it would not allow a detailed understanding of the development of the disease.

Because of the difficulty in identifying and following individual wild animals over time, most studies of chronic disease have depended on long-term studies of a population, or repeated cross-sectional surveys, rather than on the methods described above. These methods measure the occurrence of disease and the exposure to various risk factors at the population level rather than in the individual animal, and the results obtained by such studies are quite different from those obtained by the study of individuals. Assume that we wished to determine the association between exposure to a particular environmental contaminant and the subsequent development of disease. If we could follow

individuals, we would likely use a cohort method in which we would choose a group of individuals free of disease, monitor the specific level of exposure each received and then follow these individuals to determine the timing and occurrence of disease. At the completion of such a study, we should be able to make a direct correlation between the level of exposure and the rate of disease occurrence. If detailed records of exposure were available for individuals, we might choose to use a case:control method because of the economy of this approach compared to a cohort study. We would identify individuals with and without disease and then compare the level and type of exposure they had received in the past. The result of this type of study might show that individuals with the disease had received, on average, exposure to 17 units of the factor, whereas those without disease had been exposed to two units on average.

In most instances in wildlife work, we can only estimate the average exposure of the group, and the prevalence of disease by examining samples taken from the population at different times. Any associations that can be made are between the estimated average level of exposure and the estimated rate of occurrence of disease in the population. Thus, we might find that the average level of exposure in the population (determined by sampling) was 7 ± 1.2 units, and that the estimated prevalence of disease at a later time was 13%. It would not be possible to relate the degree of exposure received by any individual to the subsequent development of disease in that individual. However, this type of data could be used to compare populations with different levels of exposure to the risk factor or different prevalence of the disease.

The most difficult aspect of investigation of a chronic disease is the assessment of its significance for the population. Prevalence of a disease may be determined through cross-sectional surveys, but means relatively little without an understanding of the duration and clinical course of the disease. For example, assume that two chronic diseases, A and B, both of which are ultimately fatal, occur in a population and that the prevalence of each is 8%, based on repeated surveys. If the average length of the clinical course from onset of detectable disease to death is 6 months for A and 30 months for B, both the incidence rate and the impact on the population will be much greater for disease A than for disease B, although the prevalence is the same.

Some type of longitudinal study of the clinical disease in individuals is usually required to gather the information needed to interpret the prevalence data. In some situations, a cohort study may be possible but, in many instances, experimental replication of the disease is a much easier technique. For example, paratuberculosis (*Mycobacterium avium* ssp. *paratuberculosis*) is a chronic disease that is common in domestic ruminants and has been reported in many species of wild ruminants in captivity. Knowledge of the disease in free-living wild ungulates is limited and sometimes contradictory. There have been few reports of the disease in white-tailed deer (Libke and Walton 1975; Chiodini and Van Kruiningen 1983), suggesting that the disease is rare; however, Shulaw et al. (1986) found 2.5% of a large sample of deer to be seropositive for this disease, suggesting that exposure to the agent may be

rather common. This disease is characterized by both a long latency period and a prolonged clinical course, so that a cohort study of free-ranging animals would be very difficult. Williams et al. (1983) infected deer experimentally and found that the infected animals were small in stature and in poor body condition compared to controls, but had no gross lesions other than slightly enlarged mesenteric lymph nodes. However, the animals remained infected for the 12-month duration of the trial. The experimental results help to explain why clinical disease is recognized infrequently, even though the prevalence may be rather high, and also suggest that the disease could have a sublethal or debilitating effect. A similar combination of observational and experimental studies will likely be required for most chronic diseases.

8.3 Summary

- Disease outbreaks are often transient phenomenon. It is important to respond as quickly as possible and to go prepared to collect a variety of specimens and data while they are available.
- If possible, it is wise to necropsy a sample of affected animals in the field and, based on the results, to choose specimens for examination by a trained pathologist and other specialists in the laboratory.
- Try to establish the time of onset of the outbreak as closely as possible, and determine and map the spatial and temporal distribution of all events that might be related to the disease.
- Collect as much quantitative information as possible about the affected and non-affected portions of the population.
- Continue to monitor the outbreak regularly through its course to detect changes in the disease pattern.
- A disease may be chronic because of prolongation of the period between initiation of the process and onset of clinical disease and/or because of prolongation of the period from onset of clinical disease to termination.
- Chronic diseases, even if they produce severe clinical signs or death, may be difficult to detect because the few individuals affected at any time are removed by predators and scavengers.
- Immune function may be impaired in chronically debilitated animals; this will affect the sensitivity of tests for some chronic diseases.
- Retrospective study of records from diagnostic laboratories may provide a method of accumulating sufficient cases of chronic or rare diseases for analysis.
- Repeated cross-sectional surveys may be used to measure the prevalence of chronic disease in a population, but it may be difficult to differentiate between recently-acquired and long-standing cases. Age-specific prevalence rates can provide a measure of the incidence rate of new cases.

- Prospective cohort studies are ideal for investigating associations between risk factors and chronic disease. This technique should be considered in situations where animals can be observed or recaptured over an extended period.
- It usually is impossible to measure the degree of exposure of individual wild animals to a risk factor and then follow them to monitor the development of chronic disease. It is less difficult to measure the average level of exposure of the population and to relate this to the prevalence of disease.
- It often is necessary to use experimental reproduction of the disease to determine the clinical features and course of chronic diseases in wild animals.

9 Records and recordkeeping

"*Recording this information requires self-discipline which is unpleasant, particularly to those who feel their imagination borders on genius, but it is a necessary part of efficient research work*"

(Holman 1969)

Advances in the study of disease in wild animals, as in any branch of science, are based on cumulative experience. Each investigation is related to those that preceded it either through attempting to confirm the previous work or, more often, by building upon it. The bricks for this building process are recorded results and observations; the mortar, that allows construction, is the exchange of information among investigators. The first half of this book has dealt with collecting information and the remainder will deal with applying that knowledge to the management of disease. Hopefully these pursuits are done in a scholarly manner, i.e., in a manner befitting "*a learned or erudite person who has a profound knowledge of a particular subject*" (Nichols 2001). Four types of scholarship have been identified: discovery, integration, application, and transfer of knowledge (Boyer 1990). Hughes (1999) added the scholarship of archiving or preserving knowledge to this list. This chapter deals with preserving knowledge and is included because understanding disease is dependent upon the ability to access information from the past.

The accurate collection of data is an accepted part of any scientific endeavor but to be useful the information has to be recorded in a systematic manner so that it can be retrieved and interpreted at some later date. Much of the scientific study of wildlife disease has been concerned with short-term phenomena but it is only with accurate records that long-term trends can be detected. For example, the gradual decline in body mass, size and condition, and survival of the goslings of arctic-nesting geese, as a result of deteriorating habitat, would not have been apparent without comprehensive records collected over decades (Francis et al. 1992; Reed and Plante 1997).

The subject of keeping records for science (and particularly for investigation of disease) has received relatively little attention compared to the depth of information available on business or office record systems. In reviewing some of the books available on the latter subject, I was surprised by the similarity of the problems. For example, Aschner (1984) wrote in the preface to a book on office record keeping: "*we keep more and more files in which we can*

find less and less information". Those who have attempted the retrospective study of a disease, using records compiled by someone else, will be able to identify with this statement. In the same discussion, Aschner stated: "*You defend yourself by making an extra copy of everything and squirrelling it away in your personal filing system. Your best defense is that 80% of filed records are never called on again. Your worst fear is that one day you'll have to rely on someone else's personal filing system, and that it will be just as antiquated and difficult to unravel as your own*". Good recordkeeping is mainly a matter of discipline and habit; the intent of this chapter is to present general, common-sense guidelines that may make the process easier. As in most other specialized areas, professional assistance should be sought in designing a system and expertise from the field of business recordkeeping and data management may be valuable. However, in my experience, record and filing systems designed by individuals who do not have first-hand experience with biological problems have a remarkably short life-span. In recordkeeping, as in statistics, a balance is required between knowledge of the vagaries of nature and the absolutes of mathematics. In this chapter, the terms *records* and *data* are used in a very general sense to identify all of the information resulting from an investigation, including specimens such as those placed in voucher collections, photographs, and histologic slides, as well as the more usual written descriptions and columns of numbers.

9.1 Designing a record system

When one sets out to design a data-collection and recordkeeping system, it rapidly becomes apparent that any system one might choose is a compromise between completeness and versatility on one hand and ease of use on the other. An elaborate system that collects a myriad of detailed information may reduce some of the problems mentioned earlier with retrospective review of records. However, such a system may prove totally unusable if it is so complex that ordinary people cannot use it correctly or refuse to commit the time required to learn to use it properly. Conversely, a simple system that collects information on only a few key factors may be readily accepted and may generate an overwhelming volume of data in a few years. However, the data may be of limited use because of the failure to collect information on ambient conditions, or some basic process, at the time the seemingly more important data were collected. A guiding principle is to save what is needed and not to try to save everything.

A useful recordkeeping system also represents a compromise between the special needs and eccentricities of the individual investigator and the ability to exchange information readily with others. This feature may not be critical for the researcher who does not want to share data prematurely. It is important for those involved in the investigation and diagnosis of disease occurrences who need to exchange information rapidly in order to follow the course of disease outbreaks. The study by Brand (1984) is a good example of the use

of information for this purpose. Brand was able to assemble data from various sources to show that outbreaks of avian cholera in waterfowl from the arctic coast to the Texas Gulf Coast were not isolated events but rather a chain of occurrences along a migration corridor. The ability to exchange information quickly has become critical for investigators trying to track the occurrence and distribution of diseases such as West Nile virus and avian influenza where there is the need to provide `real-time' information useful to public health officials. Unfortunately, most of the recordkeeping systems used in the past (card files, etc.) were highly personal, so that the great bulk of information became inaccessible when the investigator moved, retired, or expired. The loss of information collected by pioneers in the field of wildlife diseases is a major factor in the general shallowness of our collective knowledge about many diseases today. It is not that these individuals were sloppy or careless in their approach to records (the reverse was often the case), but, rather that the systems available at the time were not suitable for rapid data transfer or easy recall. Retrospective re-entry and filing of such information is a laborious and expensive process that generally has been given low priority (researchers should always keep in mind that most new diseases and disease phenomena have, in fact, been seen before but may not have been recorded in a way accessible to others).

In setting out to design a data system, one must realize that whatever system is chosen it will become outdated and need revision or replacement within a surprisingly short time. Even the most advanced computer systems will become difficult to work with when the current technology changes and people have upgraded their machines and data-handling methods for the latest system. **No system is so fool-proof that it can be designed, put into service, and forgotten**. Every system must have regularly scheduled reviews, and there must be pre-emptive maintenance, rather than waiting for problems to become obvious. The preparation of an annual review of activities or an annual report that requires a complete recall of the entries for the year is a good opportunity to test the system. One advantage of computerized systems over written records is that revision can be done through programs that mate existing systems with new ones, without the necessity of total re-entry of data.

Regardless of the type of data being collected or the purpose of the investigation, certain basic decisions are necessary in designing the data-collection and recordkeeping system. These include: (i) what use will be made of the information?, (ii) who will use the information?, (iii) when will the information be needed?, (iv) what resources are needed and available to run the system?, and (v) how replaceable is the information?

9.1.1 What use will be made of the information?

The uses that ultimately may be made of information are impossible to predict, however, a different type of recording system is required for a short-term study, the results of which will be published shortly after its completion, than

for a diagnostic laboratory in which a steady stream of specimens will be examined over a period of years. In a short-term experimental study, it may be possible to record all pertinent information relating to the study in a single binder or spreadsheet devoted to the subject. In the case of the diagnostic laboratory, there may be no obvious intent to prepare publications, other than annual reports, but the accumulated results may be needed for many other purposes. One could argue that there is greater need for careful record-keeping in the diagnostic laboratory than in the short-term experiment because personnel, techniques, and situations will change markedly during the course of disease investigation in the laboratory. The more general the subject of an investigation, the broader the application of the data is likely to be. For instance, information on the geographic and temporal occurrence of a disease may be required for a variety of purposes, whereas more specialized information may be of interest only for researchers in the immediate subject area. The emergence of a number of important zoonoses in recent years illustrates the difficulty in predicting which data may be of importance. Prior to discovery of Lyme disease, intimate details of the life cycle of the tick *Ixodes scapularis* were of interest only to ticks and acarologists but now these data are critical for understanding the ecology of the disease. Similarly, most small rodents were of great interest only to specialists in small mammal ecology until hantavirus pulmonary syndrome was discovered, at which time historical data related to *Peromyscus maniculatus* suddenly became important.

9.1.2 Who will use the information?

It also is difficult to predict who might use the recorded information. The obvious first requirement is that the records must serve the needs of the investigator. In the short-term this is usually not a problem, as the investigator can generally recall the details of how and why a study was done. After several years of investigation, however, an individual will have amassed a mound of records and memory will fail. Potentially valuable data may have to be discarded because the investigator cannot remember the methods used or the conditions that prevailed at the time of the investigation. This becomes even more problematic when persons other than the original investigator try to use the information, or if several individuals, including a succession of graduate students, have contributed to the database. The aim in recording information should be to leave a record that is so complete that either the investigator or someone else can understand what was done. Every permanent record of an investigation should contain the reason for the study, a basic plan of the investigation, a detailed description of the materials and methods used, the names of persons involved and their role in the study, a list of pertinent specimens collected and their disposition, and copies of the original data sheets, as well as the results.

A major deficiency in the field of wildlife diseases has been the lack of a central database or registry of information regarding important disease events. There currently are no specific or named diseases of wild animals that must be reported to any authority and it is impossible to determine the extent of even such common diseases as botulism in waterfowl, or tularemia in rodents and lagomorphs, on other than a local basis. The databases at the National Wildlife Health Center in the United States and at the Canadian Cooperative Wildlife Health Centre (CCWHC) are models of what is required. When any new record system or database is being established, it is important to consider existing information collections on the same or related subjects, and to try to make the format compatible so that information can be exchanged easily.

9.1.3 When will the information be needed?

The normal progression during a disease investigation is that collection and analysis of data are separated in time. Often all of the data have been collected before any analysis is done. In designing a data collection system, serious thought should be given to methods that will allow data analysis to begin early in the collection period. The investigator has to worry about details and should be assessing the progress of the investigation continuously. In investigations in which the rate of data accumulation is rapid, particularly if the data are recorded automatically, the investigator may not be able to appreciate the significance of the data at the time they are collected. Consequently, errors, such as those caused by equipment malfunction, may go undetected until the information is analyzed weeks or months later. A system that allows rapid access to the data, as they are collected, should be considered, and this has become increasingly feasible with electronic technology.

Another important consideration is the length of time that data should be retained. The records from a busy research or diagnostic laboratory accumulate quickly and storage soon becomes a concern. This is an area where scientific recordkeeping differs from the systems used for business and office recordkeeping. In the office situation, the great bulk of information is required only for a short period, usually <2 years, and only a small proportion needs to be preserved longer for tax and archival purposes. Most business systems include planned disposal of senescent data. In contrast, information on disease remains pertinent for as long as it is accessible, and archival material is particularly important for detecting and measuring temporal changes. The value of museum specimens in monitoring various pollutant problems has been discussed in earlier chapters. Development of new technology, or new information, may allow new interpretation of old data. For example, "*hemorrhagic disease*" was described in Iowa muskrats by Errington (1946) and the disease was subsequently diagnosed in many areas of North America, although the cause was unknown. Karstad et al. (1971) described Tyzzer's

disease (*Clostridium piliforme* infection) in captive muskrats and suggested that the lesions were identical to those of the hemorrhagic disease described by Errington 25 years earlier. In 1979, we re-examined specimens from muskrats with hemorrhagic disease collected by Paul Errington in 1947 that had been preserved in the files of Iowa State University. We were able to confirm that hemorrhagic disease and Tyzzer's disease are a single entity (Wobeser et al. 1979a). Developments in molecular technology allow meaningful new information to be mined from old preserved materials. As an extreme example, mycobacteria have been characterized from 17,000-year-old bones of an extinct species of bison (Rothschild et al. 2001).

In designing a recordkeeping system, it may be practical to have two classes of storage: an **active** system for those records that are being used on a regular basis and for which the emphasis is on fast retrieval, and an **inactive** system for records that are used infrequently. The latter must still be accessible, but one may compromise ease of retrieval for efficiency of storage. In the past, recording of all records >3 years of age on microfilm was an example of this type of strategy. Physical specimens such as glass slides no longer needed for immediate examination can be placed in warehouse-type storage, so long as the specimens are filed systematically and are secure.

The length of a study has a direct effect on the method to be chosen for analysis and collection of data. Usually in short-term experimental studies there is no difficulty in maintaining a consistent technique throughout the study; however, in long-term studies, which may cover a period of years, there will be evolution in techniques and equipment, and changes in personnel. Consider for example that we wished to do a prospective study that involved monitoring the concentration of pesticides in tissues of every raptorial bird submitted to a diagnostic laboratory over a 10 year period. Because of changes in pesticide usage and in diagnostic techniques, it is probable that many of the methods used in Year 1 will be archaic by Year 10. During the course of the study the investigator will have to decide either to persist with the outdated methodology or to change technology. If one adopts the latter alternative, some early data may be of little value. A partial solution is to analyze specimens during the transition period to a new method by both the old and the new technique, so that appropriate correction factors can be calculated. It also is necessary to keep scrupulous records of how and when changes were made, so that it is absolutely clear in retrospect which data were generated using the old and new methods.

9.1.4 How will the system be maintained?

Entry of data and maintenance of any type of information bank are costly in terms of personnel, time and other resources. When one initiates such a program it is important to have some assurance that the support necessary for its maintenance will be forthcoming in the future. Use of computer technology

automates much of the data handling and may reduce errors, such as those made in transcribing information; however, this requires certain minimum hardware and software. In my experience, institution of an electronic data storage system is a protracted procedure that requires both considerable expertise and patience to get it functioning properly. The objective in establishing a record system should be to have it simple to use and easy to learn to use. The system should not require a great deal of time input from the disease investigator. **If the system is so complicated that it cannot be run on a day-to-day basis by support staff, it needs redesigning**. It is very easy for an investigator to become entrapped in mastering gadgetry associated with recordkeeping and lose time that would be better spent in investigation and analysis. The system should also be designed so that it will continue to function despite the inevitable personnel changes, including the computer guru who designed and implemented the system.

9.1.5 Are the data irreplaceable?

A major consideration in establishing a system for collecting and recording data is the need for hard, objective appraisal of the value or importance of the information. Although it may be costly to do so, experimental studies, by their very nature, should be reproducible. Thus, the aim of recordkeeping in this type of study should be to preserve sufficient detail to allow replication. In contrast, information relating to naturally occurring events may be irreplaceable. Because of the efficiency of predators and scavengers, together with the secretive nature of wild animals, very few sick or dead individuals are found, and even fewer are examined carefully. Data relating to such events are valuable because of their rarity and because they cannot be replicated. Every effort should be made to collect and preserve as much information as possible about such occurrences. This becomes even more important when dealing with rare or endangered species, in which each single individual becomes important. When rare specimens such as a whooping crane are found dead and become available, every effort should be made to utilize the specimen fully and to collect and preserve as much information as possible.

9.2 Logging information for retrieval

During the course of an investigation, many types of information may be collected, including field observations, photographs, maps, and written descriptions, as well as results from a variety of laboratories. This material often accumulates over an extended period of time and results of some analyses may not be available until months after the remainder of the investigation is closed. Each supporting laboratory will usually apply its own reference number

or code to any specimen they examine, and various types of data may have to be stored in different locations. The resulting plethora of reference numbers and codes can be chaotic, unless a system is established in advance to relate all aspects of a particular investigation to each other. I will describe the system we use for specimens submitted to our diagnostic laboratory, not because it is an example of advanced technology but rather because it is a simple system that works well and that can be adapted to a variety of situations. The system is based upon assignment of a single unique code-number to each animal or group of animals at the time of submission to the laboratory. We call this the **accession number**. The number for any case or specimen consists of three parts: a code-letter that indicates the submission is a diagnostic case (as distinct from an experimental case or other type), a year designation, e.g., 05, and a sequential number from a series that began with 1 for the first case of the year. Thus, a deer found dead and submitted during February of 2005 might have been assigned D-05-534, which indicates it was the 534th case submitted for necropsy in 2005. Basic information on the case, including the date of acquisition, the species of animal, the number in the group (if more than one), the name and address of the submitter, the precise location where the animal was found, the circumstance and detailed history of the case, and any pertinent reference number applied by the submitter are recorded in a permanent written log and in an electronic database at the time of submission. From this point forward, every aspect related to the investigation of the case is referenced by use of its accession number. As the results of all diagnostic tests and other pertinent information become available, these are added to form the permanent record of the case. Photographs, glass histologic slides, tissue blocks, parasites, maps, and any other information or specimens resulting from, or pertaining to, the case may be stored in different areas, but all are marked with the same accession number. This allows them to be linked back to the permanent record. Although the system was designed for a diagnostic laboratory, the basic principle of a central reference log and data base, and assignment of a sequential number to each specimen the first time it is handled can be modified for any other type of investigative situation.

9.3 Collecting information

Information may be costly and irreplaceable but one still encounters investigators who invest thousands of dollars in experiments or expeditions and then record the results on the equivalent of old envelopes and matchbook covers. I believe in the use of record sheets that are designed specifically for recording information related to a particular subject or project. The classic field notebook with blank pages is still necessary for recording unexpected and incidental information but, in most instances, it is preferable to have specific data record sheets tailored to the project. In experimental studies, one

should always assess exactly what data should be collected, and then design specific data sheets for the purpose. These are then used throughout the experiment. The major advantage of such sheets is that they automate the process. The investigator does not have to rely on memory for details of what information is required, or whether a particular snippet of information has been collected. Uniform information can be collected in this way, even by more than one investigator, and omissions or missing data are immediately evident as blank spaces on the data sheet.

The same type of data-recording sheets can be used for planned studies that involve collecting observational information. For example, during a study of the prevalence of ringworm in mule deer, we designed two specific data sheets for use by biologists examining deer at hunter check stations. The first sheet consisted of a column of pre-assigned accession numbers with space for recording the date, location of the check station, and sex and age of each deer examined. This sheet was the central log book for the check station and provided information on the total population examined at each check station. Using these sheets we could determine, for example, how many deer of a specific sex and age were examined. We provided both a written description and photographs of ringworm lesions to assist the biologists in identifying possible cases of the disease. When lesions suggestive of ringworm were seen on a deer, this was noted on the first sheet and a second form, specific to that individual animal, also was completed. Information recorded on this second sheet included the accession number from the first sheet, the general body condition of the deer, the location where the deer was killed, and the hunter's name and address. The biologist also marked the location and size of the lesions on outline drawings of the left and right side of a deer. A portion of affected skin was collected and placed in a labeled specimen bag attached directly to the second form. When the specimen and forms were returned to the laboratory, we were able to collect specific and detailed information about both the individual animal and the population from the sheets.

Generic data sheets can be used for recording information on impromptu or unexpected disease events. We have a general disease-occurrence report sheet that is used for recording basic information when we receive reports of disease occurrence. This form is very adaptable; information collected includes the date; name, address, and telephone number of the contact person(s); species and number of animals involved; location (as precisely as possible, including methods of access) and available information on the circumstances prior to and during the occurrence. Detailed information on population and environmental features discovered during a subsequent field investigation are added. The accession number of any specimens submitted to the diagnostic laboratory is also recorded. Data collected in this way not only provide information on disease occurrences but also are useful for documenting laboratory activities related to disease and for identifying individuals, such as conservation officers, who may have an interest in assisting with future disease problems. All data recording sheets of this type should be retained as a part of the

permanent record of the investigation or study, even though the information may be transcribed to other formats for analysis. If problems are encountered at the time of analysis, one can refer back to the original to detect errors in transcription or calculation.

A few common-sense guidelines are applicable when recording findings or observations:

- **always use absolute units** for describing observations and, whenever possible, express the findings numerically. For example, in describing the clinical signs of a disease, one might use terms such as: "*slightly depressed with rapid breathing*". This description has a distinct meaning to you at the time but may have a very different meaning to someone else, or even to yourself at some later date when you have forgotten the particular case. If the same animal was described as: "*indifferent to the observer and standing with the back arched, the head lowered to shoulder level, the ears held laterally and parallel to the poll, the eyes partially closed, and with a respiratory rate of 84/minute*", a word-picture is provided that is less ambiguous. Similarly, one might describe a fibroma on the skin of a deer as an "*orange-sized mass*" but, obviously, oranges come in different sizes, and "*a firm spherical mass 7 cm in diameter*" is subject to less interpretive error. Pathologic lesions are usually described in terms of distribution, size, shape, color and consistency. Similar parameters can be used for most other types of descriptive information.
- **always include the unit of measurement** when recording quantitative data. Units of measurement, as everything in science, vary among laboratories and change over time. The International System of Units (SI) is standard in most countries at present, and should be used whenever possible but units in fashion today may fall from favor, and one could be left wondering "*was that mice/hectare or mice/acre*?" or "*was lead concentration measured on a wet weight or a dry weight basis*?", unless the unit was recorded.
- **always record the actual reading or units at the time of recording results.** Various correction factors, or calculations, can be applied to the information later but the primary data sheet should contain the actual reading obtained from the thermometer, scale or other measuring device. If this simple rule is not followed, it is probable that the data will consist of a mixture of actual and corrected values that will be impossible to interpret with confidence.

The discussion above has been based on the assumption that at least some data will be recorded initially in hand-written form. Records hand-written in soft lead pencil or waterproof ink have served investigators well for many years and will continue to do so in the future. However, the same principles apply equally to other systems for recording data. Recorders have obvious advantages for situations, such as aerial surveys or behavioral observations, where the observer cannot divide his or her visual attention between

observation and writing. Completely automated recording systems may be applicable in some situations. These systems introduce the risk of machine error and require that the investigator be somewhat paranoid about monitoring machine performance to avoid loss of data (pencils seldom run out of battery power or develop a short-circuit!). A disadvantage of all of the systems mentioned to this point is that the information has to be transferred to some other system for analysis. Transcription errors may occur when the information is transferred to a storage or database management system. Written hard-copy originals have an advantage for back-checking in this regard, compared to recordings, which are often erased after transfer. Another disadvantage of all of these systems is that the data are not available for manipulation or analysis until they have been transferred to some type of data base management system. This means that it may be difficult to monitor an investigation in progress.

The use of portable computers for collection of data in the field has the advantage of combining data collection and data entry into one step. Data can be entered directly at the trap-site or beside the pile of dead ducks and transferred electronically to a data base management system where it is available for reporting or analysis. As with any mechanical system, the investigator needs to vigilant about backing-up data to prevent its loss.

9.4 Filing and storing information

The only reason for storing information, including reference specimens, is to have the material available for use at some future time. This simple fact is overlooked occasionally in the process of establishing a storage system. The most common complaints that I have heard about data storage systems relate to lost data and difficulty in retrieving information placed in a repository. In the following discussion I have assumed that most record-keeping systems will be computerized, however, many of the same general principles apply equally well to other systems. A common problem with many existing systems is that they were established based on an opportunity to acquire a hardware system. The process in many situations has been to acquire the hardware, then to see what operating systems are available, and finally to see how these could be used for recordkeeping. A little thought would suggest that the process should logically proceed in the reverse direction, i.e., analysis of the present and future needs of a recordkeeping system, then choice of the software best able to meet these needs, and lastly choice of the most appropriate hardware to run the system.

The critical factor in this entire process is the analysis of what the system is expected to do. Among the factors to be considered are:

(i) The actual functions required of the system. These will depend upon the nature of the data and what you hope to use it for. If the information is

primarily quantitative, the ability to perform statistical analyses may be paramount and one may desire the ability to recall various portions of information for comparison. In this case, any one of a number of data-base-management systems may be appropriate. If the data are primarily qualitative, such as descriptions of disease outbreaks or results from a diagnostic lab, the ability to identify and retrieve portions of data in various ways may be central and one might also require an ability to add information to the files and to generate lists or reports directly from the data base. In this case, word-processing capability with text-handling features and the ability to search for key words or phrases is obviously very important within the data-base system.

In designing a recordkeeping system for disease occurrences, one of the most important considerations is the classification system used for filing and retrieving data. One might decide that it is important to be able to recall reports based on any one of a number of parameters such as the species involved (e.g., all cases involving mule deer), location, date, and disease involved; as well as by various combinations, such as all cases involving skin lesions on mule deer that occurred in Saskatchewan in the month of March in years between 1994 and 2004. Obviously for this type of search to be possible, information on each of the variables must have been recorded. One especially problematic area is the disease classification system to be used. Several formats are available in human and veterinary medicine. These range from systems based strictly on selected diseases, in which every occurrence must be made to fit into an existing format, e.g., BOTULISM or AVIAN CHOLERA, etc.; to systems based on an hierarchical anatomical system, in which a case is filed on the basis of the organs involved, the type of lesion, and the etiologic agent. Systems of this type are often called Topographic/Morphologic/Etiologic (TME) systems. In such a system records related to an animal with renal disease might be filed under KIDNEY, NEPHRITIS, *LEPTOSPIRA* SPP. None of the existing systems is perfect. This is particularly true for diseases in wildlife, in which one is often dealing with newly described or previously unreported diseases, or with disease of uncertain etiology. An important consideration is whether related subjects should be linked together for purposes of recovery. For example, a file might contain information on 20 different parasites in ducks. It would be desirable to be able to recall those cases relating to each parasite individually but there might also be an advantage in being able to retrieve information on all cases involving nematodes, or helminths, or parasites. Similarly, it might be important to recall information on ducks in general, as well as on individual species. This type of linking of related subjects is called hierarchical filing, and establishment of appropriate linkages requires a great deal of thought and thorough testing.

(ii) The ability to modify or add to the system in the future. One must anticipate that changes will occur and that new categories will have to be added to the system as, for example, when a new disease is discovered in the area.

(iii) The anticipated size of the data base. This will determine the equipment and storage capacity required.
(iv) The need for compatibility of the database with other similar record systems, so that information can be exchanged readily. One of the most exciting possibilities for the study of wildlife disease is the incorporation of information relating to disease into so-called geographic information systems (GIS) for spatial analysis. The GIS is a software system that stores, organizes, retrieves and analyzes data according to spatial relationships, and allows automation of map construction to demonstrate spatial relationships. This technique has great promise for epizootiologic studies, so long as spatial information about disease is recorded in a format and detail compatible with other information, such as land use, stream location, topography, human demography, climate and weather data, stored in other databases.
(v) The physical type and configuration of the records. In our diagnostic laboratory, all materials relating to a particularly accession are linked together by a single code-number, as discussed earlier. The history of the case, together with all laboratory findings, is recorded in an electronic database. Photographs relating to the case are maintained in a separate file in which cases are arranged in sequential order by the same numbering system. As this is written we are in transition from film photography to digital photography, which requires an adjustment to the fact that photos are stored in an electronic data base (that requires a search program) and not in a physical file where one can leaf through them to find the image required. Histologic slides, paraffin-embedded tissues, tissues for electron microscopy, etc. also are maintained in separate files. Thus the filing system actually consists of a number of files located in different areas, all arranged in the same manner and linked by a common number. In our system, a search via computer for a particular subject, e.g., mule deer with ringworm, would yield a list of accession numbers which could then be used to find appropriate information or specimens in the various files.
(vi) Ease of data entry and recovery. The investigator should prepare a list showing exactly what he expects from a filing system and take this, together with representative samples of the type of data to be included, to specialists in data-filing systems. One should not begin to consider hardware systems until after the requirements for the system have been established. One should approach the institution of a new filing system with the same care and consideration applied to marriage. Mistakes as a result of infatuation can occur in both cases and the maxim act in haste and repent at leisure is equally true for both.

Once a system has been chosen and established, a few general guidelines are appropriate. First, **one person should be responsible for management of the system**. This individual should oversee the training of others who use the system

and should ensure that abuse does not occur. He/she also should be responsible for monitoring the system for problems and for making changes or additions, as required. It is very important to control who can change the program, and under what circumstances this can occur. Second, **the central data file**, whether in the form of a computer bank, card file, or filing cabinet full of written documents, **should be treated as irreplaceable**. Material should never be removed from it and original materials from it should never be on or in an individual's desk. Instead, material from the central file should be copied, and the copy used. Third, **all files should be backed-up continuously** to prevent loss of information through some natural or other disaster. The process of backing-up data applies to all stages of information collection and storage and, even in the field, the day's field notes should always be transcribed to a permanent log each evening, so that loss of a record sheet or note-book results in minimal loss of data.

At this point I must confess to being somewhat of a Luddite or perhaps overly skeptical about the long-term reliability of electronic storage systems. I fully realize the advantages that these systems offer, but the rapid pace of technological advancement may result in data collected and stored even a decade ago becoming difficult to recover, unless a conscious effort has been made in the intervening years to consistently update the data each time a new improved version of the software becomes available. Earlier, I related the story of being able to go back in 1979 and read the handwritten documents related to cases of hemorrhagic disease submitted to a diagnostic laboratory by Paul Errington in 1947. I wonder if anyone in 2037 will be able to recover the electronically preserved records on a bald eagle that I examined on November 2, 2005 (assuming, of course, that someone would want to do so!).

9.5 Specimen collections

Physical specimens, such as serum, histologic slides, parasites and other disease agents, as well as photographs collected during an investigation are a part of the record of that study and should be retained in the same manner as other types of information. The factors that must be considered relate to the value of the materials for future investigations, availability of methods for preservation and storage of perishable materials, and the cost of preservation and storage. The latter factor has to be balanced against the ability to replace the material and the cost of replacement. In addition, each type of specimen has individual characteristics that may make it more or less valuable. Often the full value of a specimen may not be apparent at the time of preservation. For instance, cryogenically preserved deer mice in two university collections provided material to prove that Sin Nombre virus, the cause of hantavirus pulmonary syndrome in humans, had been present for many years prior to the first recognized human case (Yates et al. 2002). New technologies, such as polymerase chain reaction and other molecular techniques, have made it

possible to identify disease agents in very old specimens, including identification of *Mycobacterium* spp. DNA in bone from an extinct species of bison (Rothschild et al. 2001).

Serum is perhaps the most easily acquired and versatile type of specimen available for the study of disease. A single sample can be used to test for antibodies to a variety of infectious agents, as well as to assay the concentration of hormones, trace minerals, toxins, enzymes and other substances. Wildlife disease investigators often have the opportunity to collect blood samples as part of studies or incidental to other activities, such as trapping and translocation. In most instances, when samples are collected, only a small portion of the sample is used for a specific purpose and the remainder is discarded, rather than stored. The value of serum collections or banks is well established in human and veterinary medicine but the concept has received little attention in the area of wildlife diseases. Collections of serum may vary from those collected over time from a small local population of animals being followed in a long-term study, to major national or international banks for which specimens are collected according to a planned random sampling scheme, so as to be representative of the population under consideration.

The general objectives in establishing a serum collection are to have a source of documented material for contemporary or short-term studies (e.g., surveys of disease prevalence or distribution), and as a repository for samples that may be used for retrospective studies (Moorhouse and Hugh-Jones 1981). Retrospective studies might include investigation of a newly discovered disease (to determine if it was present before it was recognized), a disease for which the cause has been discovered recently, or where new techniques for assay of some variable have become available, and for monitoring temporal changes in a disease or the effectiveness of a control program. For example, Docherty and Romaine (1983) used serum collected over several years from a population of captive cranes to determine that the population had been exposed to the herpesvirus causing inclusion body disease of cranes for at least 2.5 years prior to the first recognized mortality caused by the agent. This discovery led the authors to suggest that overcrowding and inclement weather were risk factors in the occurrence of mortality.

The limitations of serum collections are related to the sampling procedure used to assemble the samples, stability of the various components in stored sera, and the documentation available on the source of the samples. Sampling procedures for national veterinary serum banks, that are representative of the population of cattle or other species under study, have been discussed by Moorhouse and Hugh-Jones (1981) and Kellar (1983). The advantages, disadvantages, and biases of samples collected in various ways were described in detail in these reports. No such systematic sampling has been applied to a wild species to my knowledge and many sources of serum from domestic animals, such as the slaughter house, are not applicable to wild species.

Many samples from wild species are likely to be available as by-products of other studies, e.g., when blood samples are collected during translocation

projects or during outbreak investigations. Although such samples may not be totally representative of the population and may be subject to a variety of types of bias, they are better than no samples, and may be very useful. For example, Thomas et al. (1984) examined sera collected from coyotes, trapped for other purposes in three states between 1972 and 1983, for antibodies to canine parvovirus-2. No antibody to the virus was found in samples collected prior to 1979 but, beginning in that year, there was a rapid increase in the proportion of positive sera from all three areas, and >70% of sera collected in 1982 contained antibodies to this virus. This sudden onset of exposure to the virus coincided with the recognition of clinical disease caused by the agent in dogs and provided strong evidence for the emergence of canine parvovirus-2 as a new disease in the coyote population. A major advantage of combining serum collection with other activities is that a bank of samples can be collected over time with little added expense other than that for the tubes used in the field and the cost of storage of the samples. Consider the costs that would have been involved had Thomas et al. (1984) set out to capture the 1,184 coyotes used in their study. In our experience, researchers and field personnel are very willing to collect blood samples if they are provided with tubes, needles and a clear, concise protocol for handling specimens. We also have had success collecting blood samples from deer by providing hunters with tubes and an instruction sheet prior to the hunt, and having the hunters return the filled tubes at hunter check stations.

The stability of various immunogobulins in serum has been reviewed and specific recommendations regarding storage methods have been made by Moorhouse and Hugh-Jones (1981). Lyophilization (freeze-drying) may be the method of choice for long-term storage but freezing has proven satisfactory in many laboratories. If the size of sample permits, the serum should be sub-divided into small aliquots prior to storage so that a portion of the total sample can be used without consuming the entire specimen. A very important consideration in establishing a collection of any type is to determine in advance who will have access to the specimens and under what circumstances the banked material will be withdrawn. This is particularly important when the collection is based on donations from many individuals. If individual serum samples can be subdivided into aliquots, consideration should be given to retaining one aliquot of each specimen as a permanent reference, to be used only under exceptional circumstances. Use of the other aliquots can be based on the scientific merit of the proposed study and several studies should be run in tandem when a vial is opened for use. A requirement should be that results of all tests performed using material from a collection must be returned to the collection for inclusion in its permanent records.

Serum samples, as any other biological specimen, are of very limited value without proper documentation. The minimum information that should be available for every specimen stored in a collection is the date of collection, the species, sex and age of the animal, and the location. Special information, such as detectable disease in the individual, recognized disease in the population, and method of capture/handling of the animal also should be included. The

information should be filed so that the contents of the collection, details of deposits and withdrawals, and the results of tests performed on samples from the collection are readily available.

Retention of biological specimens of other types as part of the record of an investigation depends very much upon the individual circumstances and facilities available. For example, we have been able to retain all of the paraffin and plastic-embedded specimens collected during the past 40-odd years of investigation of spontaneous diseases in wildlife. Only a small proportion of this material has been reused to date but, when required, it has been very valuable. As storage space has become filled, we systematically discard glass histologic slides, beginning with the oldest, but we retain paraffin-embedded tissues, so that new slides can be prepared if required. We have not attempted to retain all of the parasites, viruses or bacteria encountered during the same time. This reveals my bias as a pathologist but it is also a recognition of the difficulty in retaining perishable materials. Whenever possible, representative specimens should be retained. The requirement by many periodicals, such as the Journal of Wildlife Diseases, that voucher specimens of parasites be deposited in a recognized museum or collection prior to publication of results, is an added incentive for this process.

A camera is one of the most important and versatile tools available for preserving a permanent record of disease investigations in both the field and laboratory. Photographs are almost indispensable for communicating the results of investigations to others, particularly to the public, and this aspect of the job of disease investigation should not be underestimated. Often the success of a project rests on one's ability to interest others, and to convince them of the project's merit. Good photographs can be very persuasive in this effort. In field investigations, photographs are a convenient way of recording general habitat conditions as well as specific details related to disease events. The camera often records information that was not obvious to the investigator at the time. On several occasions, while reviewing photographs, I have recognized significant environmental features, characteristics of animals, or examples of sloppy technique on the part of myself or others that were not observed at the time the photographs were taken. Easily portable, pocket-style cameras can be taken anywhere in the field and provide adequate photographs for most situations. In the laboratory, more sophisticated systems may be used to prepare publication-quality photographs. A few general guidelines are appropriate in both situations. Photographs of specimens should contain a reference or scale, so that size may be determined later. A reference number or some other code also should be included, so that one can relate the resulting photographs directly to a particular case or investigation. Negatives, in the case of black and white photographs, and the original copy in the case of color transparencies, should be maintained in a permanent file, so that pictures or copies are available as required. It appears that techniques and protocols for long-term storage and retrieval of digital images are still in the development stage and not in general use, so that many photographs

are being taken without much thought about how these images will be retained in a permanent file (other than by an individual) or retrieved. Video cameras have received relatively little use but are particularly useful for recording clinical features of disease, as well as for producing materials that may be useful for teaching and explaining the nature of wildlife work to the public.

Record and specimen collection and storage are an often neglected area in scientific studies. These aspects should receive the same degree of planning that is devoted to other parts of the methodology, with particular attention devoted to defining how, and by whom, the materials will be needed in future.

9.6 Summary

- Recordkeeping systems are a compromise between completeness and ease of use. The goal should be to save what is needed and not to attempt to save everything.
- Record systems are like a garden, in that both require continual attention. Data bases become outdated quickly and require continual maintenance and updating. Any change made to the system must be recorded scrupulously, so that it is immediately evident which information was collected under the new and old systems.
- Important factors to consider in designing a system include uses to be made of the information, identity of the users, timing of use, ease of data entry and retrieval, resources required to establish and run the system, and the value (replaceability) of the data.
- The process of choosing an electronic system should progress from analysis of present and future needs, to choice of software able to meet these needs, to choice of hardware to run the system.
- A simple system based on a sequential numbering system for each new case, outbreak, or occurrence has wide application in disease studies.
- All information should be recorded in absolute units and the units used must be recorded on the data sheet. Record sheets that are specific to the study automate the process of data collection and help avoid errors.
- One person should be responsible for managing, monitoring, and revising the system. However, procedures must be sufficiently well documented that the system will not collapse with a change of personnel. All original material in the files should be treated as irreplaceable; originals should never be removed, except for copying followed by immediate return.
- Because electronic data management systems evolve so rapidly, scrupulous attention must be paid to updating files so that information does not become inaccessible after a number of updates.
- Specimen files or collections, such as serum banks or helminth collections, provide reference material for current studies and allow retrospective investigation using new technologies. Advance planning is required to determine how, when, by whom, and under what circumstances, these specimens will be used.

Section III
Disease management

"*We would do well to remember that management is always a delicate thing, demanding skill and sensitivity rather than a formula. . . .*"

(Herman 2002)

10 Disease management—general principles

> "*The last century was one of triumphs and failures. The triumphs came mostly in the first 70 years of the 20th century: they resulted primarily from understanding the ecology of certain diseases through field and laboratory research and then using that knowledge to develop and implement prevention and control programs aimed at breaking the transmission cycles at their weakest points. The failures occurred when we became complacent after successes were achieved and relied too much on the 'quick fix' or the 'magic bullet' approach to disease control*"
>
> (Gubler 2001)

Discussion to this point has dealt with investigation of disease in wild animals. One basic reason for studying disease is to assess its significance and, if necessary, to identify methods by which the disease might be influenced in a manner considered to be beneficial. If we distill disease management to its most basic form, there are only two options: reduce exposure of the animals to the causative agent or factor, or reduce the effect of the factor or agent on the animals (in some instances, such as the introduction of myxomatosis and rabbit hemorrhagic disease into Australia, the opposite effect may be desired). The remainder of this book will deal with methods for the management of disease in free-ranging animals, and management will be considered in the sense of to restrict or to curb the occurrence or the effects of disease.

10.1 Is management desirable?

A question that must be dealt with immediately is the philosophical and ecological desirability of attempting any type of disease management in free-ranging wild animals. All disease in free-living animals is considered by some people to be a natural phenomenon that contributes to the 'balance of nature'. Marcogliese (2005) presents reasoned arguments that support the view that "*healthy ecosystems have healthy parasites*" and Horwitz and Wilcox (2005) note that "*parasitism is not simply a pathogenic relationship requiring treatment, but rather a process that through multiple agencies contributes to within and between species diversity, community structure and diversity, and therefore the ability of organisms to respond to change*". This

point of view reveals a clear difference between the way disease is viewed in humans and domestic animals on one hand, and in wild species on the other. Very few people would argue that smallpox or other great plagues should be allowed to run their course in human populations, although these diseases may be powerful selective forces for the continued evolution of our species. To allow some of these diseases to go unchallenged, when we have the capacity to influence their course, would be to promote selective genocide of those in less developed parts of the world. Similarly, there is little sentiment for allowing foot-and-mouth disease to become established in North American cattle or to leave pet dogs unprotected by vaccination against canine distemper. In contrast, attempts to manipulate disease in wild species may be viewed as unnatural interference. If we accept this reasoning, an investigator may be motivated by a sense of curiosity to study the role of disease in the population biology of a species, but should not intervene to change the course of the disease. In considering this question, we should examine the rationale for disease management. The reasons for undertaking any type of disease manipulation in wild animals are essentially anthropocentric, i.e., management is usually done to benefit humans in some way. Since this is the case, a hands-off approach may be entirely appropriate where the consequences of disease are perceived to be of little or no consequence, and we can act as disinterested observers of what happens to the animals. This approach would be even more appropriate if the animals were unaffected by human activities and lived in a pristine environment where disease was a truly natural event. Undoubtedly, disease in the general sense is a natural phenomenon and animals became sick and died of a variety of diseases under pristine environmental conditions. However, **every** wild animal now lives in an environment modified to some degree by humans. Many of the environmental changes that have occurred have been undesirable and have altered both the type and manner in which disease occurs. For example, the enhanced accumulation of cadmium in the organs of wild moose as a result of acid precipitation (Froslie et al. 1986; Borg 1987), selenium poisoning of water birds using irrigation runoff water (Ohlendorf et al. 1988), and the introduction of exotic diseases such as avian malaria to Hawaii (Warner 1968; Van Riper et al. 1986), trichomoniasis to the Galapagos Islands (Harmon et al. 1987), *Elaphostrongylus rangiferi* into caribou in Newfoundland (Lankester and Northcott 1979), and West Nile virus to North America are not natural events. Each of these resulted from human activities. If one appraises the situation critically, most disease-management activities in wildlife are actually exercises in mitigation. The manager usually is trying to soften or reduce the effect of some other human activity.

As the amount of natural habitat available for wild animals has decreased, the impact of humans on wild species has increased. The last half of the 20th century saw "*the most rapid period of large scale ecological transformation in human history*", with "*the disassembly of orderly natural communities*" (Wilcox and Gubler 2005). For some wild animals, the intensity and the level of sophistication of management have also increased remarkably during this

period. This is perhaps most evident for wild waterfowl in North America. Many wild ducks are now hatched in nests on artificially created islands or in artificial nesting structures located on wetlands created by damming runoff water from agricultural land or urban areas. The mallard, northern pintail and Canada goose (as well as many other species) derive a substantial part of their nutrition from agricultural crops, and special feeding sites or lure crops are used to concentrate the birds and reduce their depredation on farmers' fields. Many individuals of some waterfowl species begin life in an incubator or captive facility and then are released to the wild to 'supplement' natural reproduction. The birds face human-made hazards, such as pesticides, overhead wires, domestic pets and hunting regularly. They winter on intensively managed refuge areas that promote the artificial concentration of large numbers of birds on small areas for a prolonged period of time. Such circumstances can hardly be considered natural. Disease management may need to be a part of the integrated management of these species, in the same way that disease management is an integral part of intensive animal agriculture. The need for disease management is even more evident for endangered or threatened species where a single disease occurrence could extirpate the remnant population and where managers are forced into the use of practices such as translocation and captive breeding, which enhance the probability of disease occurrence. Leopold (1939) left little doubt of his opinion of the importance of disease control when he included a chapter on the subject in his seminal book on wildlife management and stated: "*In its more advanced stages, game management is in effect the art of maintaining a population which is vigorous and healthy in spite of its density*". What is needed is management that is based on an understanding of ecosystem level changes caused by humans and that seeks to reduce but not over-control the effect of disease agents (Horowitz and Wilcox 2005).

10.2 Is management feasible?

A second basic question that needs to be addressed is the feasibility of management of disease in wild animals. If management is impractical, there is no benefit in proceeding further with this discussion. Skeptics sometimes dismiss the study of disease in wildlife as an interesting but esoteric pursuit because, as has been stated to me: "*even if you find a disease you can't do anything about it*". This attitude is largely a result of tunnel vision, in which disease management is perceived to occur only through the medication and treatment of sick individuals. The skeptic "*overlooks the obvious fact that "doctoring" is of recessive importance in health control, even in domestic species and man*" and that "*the real determinants of disease mortality are the environment and the population*" (Leopold 1939). Treatment of sick individuals and immunization are important components of human and veterinary medicine,

but most of the real advances in general health of both humans and domestic animals are attributable to management of environmental factors, such as nutrition, provision of safe drinking water, adequate shelter and sanitation, and through regulation of population density. The best proof of this is the rapid deterioration of health and the emergence of pestilence that occurs in human populations in time of war, when there is environmental and social disruption. Similarly, most disease problems in livestock occur among over-crowded, poorly managed animals. Environmental and population factors that influence disease can be manipulated in wild animal populations and wild animals "*are being "doctored" daily, for better or for worse, by gun and axe, and by fire and plow*" (Leopold 1939), but it is interesting that improved sanitation, nutrition, and provision of clean water seldom have been included as part of disease management in wild animals.

A basic feature that affects the feasibility of management is the ability to detect and monitor changes in the occurrence of disease. There are many aspects to detection including:

- how quickly will a new disease be detected?
- how far will it have spread and how entrenched will it be before it is detected?
- how accurately can the disease be detected in the individual animal, i.e. how sensitive and specific are the tests, and how wide are the confidence interval about estimates?
- what proportion of the population can be monitored?
- how often can the population be monitored?

10.3 Who is management for?

If one can decide that management of a disease is acceptable and at least potentially possible the next step is to assess whether or not it is needed, i.e., why is the program being undertaken? As noted earlier, the rationale for disease management is almost always based on an anthropocentric view of the world and the three major reasons advanced for management of disease in wild animals are that the: (i) presence of disease in wild animals is a threat to human health, (ii) presence of disease in wild animals is a threat to the health of domestic animals, (iii) disease is having a significant deleterious effect on the population of a wild species considered beneficial to man. I placed these three reasons in this order intentionally, because it represents the real world priority for disease management in wild animals. There is more interest in the role of wild animals in human disease at present than at any time in the past, because of the global problem of emerging infectious diseases in humans. Most of these diseases are zoonoses and many are associated with wild species.

It is very important at the outset of any proposed management program to identify the **target population**, i.e., "*the population of concern or interest*" (Haydon et al. 2002). For most zoonotic diseases, the target species is *Homo sapiens*. The billions of dollars that have been spent worldwide to manage rabies in wild carnivores have been committed to protect humans and not to benefit foxes or raccoons. In other situations, domestic animals are the target, e.g., management of bovine tuberculosis in badgers, brushtail possums and white-tailed deer is done for the benefit of cattle (and humans) and not for the wild species. Occasionally a wild species may be the target population; for example, programs to vaccinate domestic dogs have been designed to protect the Ethiopian wolf from shared diseases (Laurenson et al. 1998). Identification of the target species is important because the ultimate success of any management program must be based on the effect on the target species. For instance, a program that reduced the prevalence of tuberculosis in deer in an area but did not reduce the incidence of the disease in cattle would not be considered a success.

Disease management might be attempted for other reasons, such as when disease decreases the value of a wild species for human use, e.g., when the presence of parasites makes game meat aesthetically unpleasing, or when a skin disease mars the value of the pelt of a fur-bearer; when the occurrence of disease creates a nuisance factor or raises concern among the general public; or when the presence of a condition in wild animals indicates a degree of environmental degradation that is unacceptable for human health. In the latter instance, the wild species acts as an unintentional monitor of overall environmental health. The assessment of the risk from disease in each of these situations (threat to human health, threat to domestic animals, or negative effect on the population of wild animals) requires detailed quantitative information on the occurrence and effect of the disease in the wild population, and of the probability of spread from the wild animals to humans and/or livestock, where appropriate. This, in turn, requires that there are accurate methods for diagnosing the disease, and for surveillance of the disease agent and of its effects in all relevant populations.

It is usually much easier to define (and defend) the rationale for management of a disease that affects human or livestock health than it is for diseases that are restricted to wild animals. This partially reflects the difficulty in assessing the population effect of disease in wild animals. In some instances, such as catastrophic die-offs that have occurred in bighorn sheep throughout North America, it may be relatively easy to demonstrate a need for management. But, when biologists cannot agree if the effect of hunting and natural mortality on waterfowl populations are additive or compensatory (Johnson et al. 1997; Pöysä et al. 2004), it will be very difficult to prove that management of a disease, such as botulism, on a single marsh would have any detectable effect on the overall population (although it might be important for a local segment of the population). Samuel (1992) presents a good discussion of the difficulty in assessing the effect of disease on a waterfowl population.

10.4 Costs and benefits of management

Discussion of the rationale for any disease-management program should include some type of benefit:cost analysis. This analysis should include consideration of the relative merit of various methods of meeting the same objective. Consider a marsh in which botulism kills a number of ducks every year. The overall objective of the waterfowl manager responsible for this marsh might be to produce more ducks, and the measurable end-product of his efforts is the number of ducks that are alive in the autumn. Among the strategies available to the manager to meet this objective are to: (i) increase natality (i.e., produce more ducklings and accept that a proportion of these will die of botulism), (ii) to reduce mortality to all causes, including botulism, or (iii) some combination of (i) and (ii). The cost of various types of management to increase waterfowl production, such as through construction of nesting islands and nesting structures, and of methods to reduce mortality as a result of predation, have been estimated in terms of dollars per duckling produced (Lokemoen 1984; Chouinard et al. 2005). I am not aware of any similar attempt to estimate the value of a mallard saved from botulism, either through treatment of sick birds or preventive management. This type of analysis will be necessary if disease management is to be accepted as a valuable practice.

Assessment of the costs of management must go beyond the short-term monetary costs and must consider long-term ecologic costs and benefits that may result from management (many of these may be unexpected and, hence, impossible to assess in advance). Examples of potential long-term costs include: (i) development of resistance to treatment as a result of the intense selection pressure placed on a disease agent (treatment may be successful in the short-term, but fail in the long-term, and resistance may be to more than one therapy or treatment); (ii) development of resistance in species other than the one being managed, so that new problems emerge; (iii) loss of components from the environment, such as predators and parasites that limit the abundance of the disease agent, so that the population of disease agents increases; (iv) selection for new dispersal and transmission methods that allow the agent to find hosts; and (v) changes in abundance of a species as a result of removal of one disease as a limiting factor, that allow emergence of other disease problems. As an example of the last point, fox populations have increased dramatically across Europe and foxes have become common in urban environments, at least partially because of elimination of rabies by vaccination (Chautan et al. 2000). This has resulted in emergence of *Echinococcus multilocularis* as an important zoonosis (Sréter et al. 2003) and the increased fox population also has raised concerns that if rabies reappears there may be larger and more intense epizootics (Smith and Wilkinson 2003).

10.5 How will management be done?

Selection of the most appropriate method for management requires a clear understanding of the cause and ecology of the disease and of both the course of the disease in the individual and the biology of the disease within the population. Disease management can be viewed as a tactical battle in which one uses intelligence gathered about the disease to identify the most vulnerable point at which to attack. The soft underbelly of infectious diseases is often in the method of transmission and many techniques are designed to interrupt the process or to prevent contact between agent/causative factor and host.

In every disease-management scheme it is imperative to identify all the players and to clarify their role. We have already discussed the need to identify the target species for whom the management is being done. In non-infectious diseases, such as intoxications caused by environmental contaminants, it usually is relatively easy to identify the species involved and the source of the toxicant, which may be from a discrete point or more diffuse source. The next step is to identify how the material reaches the target and this may involve other species. For example, cadmium poisoning of badgers in the Netherlands involves bioaccumulation of cadmium in earthworms eaten by badgers. The rate at which this bioaccumulation occurs depends upon the soil pH, which is influenced by acid precipitation (Klok et al. 2000). In some infectious diseases, only the target species is involved and in these it may be possible to manage the disease without considering other sources. However, most important infectious diseases that involve wild animals involve some source or reservoir outside the target species. The reservoir may be one or more animal species or some abiotic feature of the environment. Haydon et al. (2002) defined a reservoir as: "*one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population*". As an example, the reservoir for anthrax is soil, within which *Bacillus anthracis* can persist for years. In some diseases, species other than the target are an obligate part of the life cycle of the disease agent, as is the case with many diseases caused by helminths, arthropods and protozoa. Other species also may be involved in disease transmission without being an obligate part of the agent's life cycle, e.g., pox viruses may be transmitted among birds on the mouthparts of mosquitoes. The alternate or other species may be infected with or without having recognizable disease.

If more than one vertebrate can be infected by a disease agent, the condition is often called a multihost disease. In multihost diseases, it is critical to define the role of each vertebrate host species as an early step in planning management. **Maintenance hosts** are those in which the disease agent is capable of cycling independently within the population in the absence of an external source of infection. **Spillover hosts** are those in which the disease can be

transmitted within the population but in which the disease will die out without an external source of infection. **Dead-end hosts** are those in which the disease is not transmitted within the population and all infections result from an external source (Caley et al. 2002). If the objective of management is to reduce transmission of the disease, it is important to direct actions primarily at the maintenance hosts. For example, the nematode *Heterakis gallinarum* is a multihost parasite that occurs in ring-necked pheasants, grey partridge and red-legged partridge. The pheasant is a maintenance host for the worm, while the partridges are spillover hosts, although the grey partridge may be affected severely by the parasite (Tompkins et al. 2002). In this situation, management would be most profitably directed at the infection in pheasants. Feral pigs were found to be a dead-end host for *Mycobacterium bovis* infection in Australia and the prevalence of tuberculosis in pigs declined following destocking of cattle and water buffalo, without any attempt to direct manage the disease in pigs (McInerney et al. 1995). In some situations, management can be directed at preventing exposure of a spillover or dead-end target species to the disease. For instance, humans are a dead-end host for rabies in most situations, but education can be used to reduce exposure of the public to rabid animals that are the maintenance host for the virus.

Even within a single species it is important to identify those individuals that are responsible for most of the transmission, because these are the logical focus for management. Woolhouse et al. (1997) examined a range of infectious diseases and found that "*typically, 20% of the host population contributes at least 80% of the net transmission potential.*" They suggested that management directed at the 20% group is potentially highly effective, while programs that fail to reach all of this group are likely to be much less effective in reducing disease prevalence in a population. The so-called '20/80 rule' appears to apply in several diseases of wild rodents. In yellow-necked mice, sexually mature males with high body mass are the segment of the population responsible for the majority of transmission of tick-borne encephalitis virus infection (Perkins et al. 2003). Adult males also were responsible for most of the transmission of an intestinal nematode in yellow-necked mice (Ferrari et al. 2004). The reservoir for hantavirus infection in rodents is thought to be long-lived, persistently infected individuals, particularly adult males (Calisher et al. 2001). In each of the above examples, adult males appear to be the segment of the population that deserves attention. Skorping and Jensen (2004) went further and suggested that, as a general rule, those interested in disease management in mammals need to look particularly closely at males. Different types of disease demand different strategies. For non-infectious diseases, such as various types of intoxication, management is usually directed at either eliminating the source of the risk factor or at limiting access by animals to the risk factor. The occurrence of many such diseases appears to be relatively independent of host population density.

Management of infectious diseases is complicated by replication of the causative agent, with transmission to other susceptible individuals in the population.

An extensive literature has emerged on theoretical aspects of the population biology of infectious diseases. Application of these concepts to disease in wild animals is exemplified in Hudson et al. (2001). The reproductive or transmission success of the agent appears to be central in determining the type of management that may be most appropriate. Often this parameter is expressed as R_0, the basic reproductive rate or number of the disease agent, which measures the inherent transmissibility of the agent (Fraser et al. 2004) and is equivalent to the intrinsic rate of increase (r) in population models. R_0 is not a constant for a particular disease agent; it is determined by features of both the agent and the animal population in which it occurs.

10.5.1 Microparasites and macroparasites

Infectious agents can be divided into two groups on an ecological rather than a taxonomic basis (Anderson and May 1979). **Microparasites** (viruses, bacteria, protozoa) are characterized by small size, short generation time, and the ability to multiply directly and rapidly in the host. **Macroparasites** (helminths, arthropods) have much longer generation times and direct multiplication within the host is absent or occurs at a low rate. The type of disease produced by the two groups is quite different. Microparasites usually produce short-term transient infections (in relation to the life-span of the host) and induce long-lasting immunity to reinfection. Macroparasites produce persistent infections with continual reinfection, and both the immune response and the pathology produced depend on the number of parasites harbored by the host. R_0 for microparasites has been defined as the "*average number of secondary infections attributable to a single infectious case introduced into a fully susceptible population*" (Fine et al. 1982). R_0 for macroparasites is defined as the average number of female offspring that live to reproduce produced by a single female introduced into a completely susceptible population, or "*the number of 'next generation' adult parasites that would arise from one adult parasite in a totally susceptible population*" (Roberts et al. 1995). In either case, when $R_0=1$, an infection is barely able to maintain itself in an enzootic state. If R_0 falls to <1, the incidence of disease will decline, eventually to extinction. The aim of management programs for most infectious diseases is to depress the reproductive rate and, for eradication, it must be reduced and maintained below 1 (Anderson 1982). In general, diseases with a high reproductive potential will be more difficult to control than those with a low R_0 value (Anderson 1982).

The features of micro- and macroparasites may have a great effect on the type of management measure that is most appropriate. For example, immunization may be much more appropriate for the control of a disease caused by a microparasite than for a disease caused by a helminth. In the latter case, management might be directed at decreasing the burden of worms in certain heavily infected individuals in the population, rather than

preventing infection. Although the basic reproductive rate is not known for most infectious diseases of wild animals, models have been constructed that estimate threshold populations required for the occurrence of epizootics of certain diseases. For example, the population density of foxes required for maintenance of rabies in some areas of Europe was estimated, on the basis of both epizootiological observations and modeling, to be approximately 1 fox/km^2 (Anderson et al. 1981). Such estimates and the modeling techniques that have developed provide a theoretical basis for planning programs.

10.6 A management matrix

Disease management can have one of three broad objectives: prevention, control, or eradication.

Prevention includes all those measures designed to exclude or prevent the introduction of a disease into unaffected animals or into an unaffected population, and these can be applied at either the individual animal or the population level. Examples range from restrictions on the importation of exotic animals to prevent the introduction of foreign animal diseases such as foot-and-mouth disease into a geographic area, through procedures such as fencing to limit exposure of animals to toxins or infected animals, to protection of animals within a population through immunization.

Control, in the narrow sense, applies to activities designed to reduce the frequency of occurrence or the effects of existing disease within a population to some acceptable, or perhaps more accurately to a tolerable level. Often this level is defined by the funding available for control activities and by a point where the cost of further control outweighs any additional benefit that might be derived. By definition, disease control implies that some level of disease will persist in the population and this means, in most instances, that the control measures will have to be continued in perpetuity with continuing costs.

Eradication involves total elimination of an existing disease and often requires a Herculean effort. The term eradication has been used in many ways. Yekutiel (1980) proposed that eradication is "*the purposeful reduction of specific disease prevalence to the point of continued absence of transmission within a specified area by means of a time-limited campaign*". Ottesen et al. (1998) proposed that eradication is "*Permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts* . . ." and differentiated this from elimination of disease ("*Reduction to zero of the incidence of a specified disease in a defined geographic area* . . .") and elimination of infection ("*Reduction to zero of the incidence caused by a specific agent in a defined geographic area*. . . ."). The definition by Yekutiel is appropriate for our purposes because it includes the elements of space and time. Only one infectious disease (smallpox) has

been completely extinguished as a free-living agent as a result of a management program; however, many diseases have been eradicated on a regional basis. Eradication usually is contemplated only for the most serious of diseases, but there have been successful localized disease eradication programs in wild animals. Foot-and-mouth disease was successfully eradicated from deer in California in 1923 (Brooksby 1968) and mercury poisoning of birds (associated with the use of alkyl-mercurial seed-dressing agents) was eliminated from Sweden (Wanntorp et al. 1967). The present effort to eliminate the use of lead shot for waterfowl hunting in many areas of the world is an attempt to eradicate lead poisoning in wild waterfowl. Eradication programs have a finite end-point and, if accomplished, the emphasis usually shifts to prevention of reestablishment of the disease, without the recurrent costs for control.

The choice among these three basic techniques depends upon many factors including the presence or absence of the disease in the area, the length of time the disease has been present, the frequency of occurrence and distribution of the disease, the species affected, the availability of suitable methods for detection, diagnosis and management, the desirability or need for management, and the ability to convince others of this need. Often an overall program may involve aspects of prevention, control and eradication, with different techniques being used at various stages of the program.

Management may be attempted through manipulation of any of the three basic determinants of disease: the agent, the host, or the environment. Influencing human activities may be considered as management of the host or environment, depending upon the features of the disease. If we combine the three objectives mentioned earlier with these three potential sites for manipulation, we can construct a matrix of management possibilities:

	Agent	Host	Environment
Prevent			
Control			
Eradicate			

Some agents or risk factors can be prevented entry to an area, and other risk factors, such as certain toxins, may be reduced or eliminated. The host population may be manipulated through reduction of population density, dispersal from areas where the disease occurs, or by increasing the resistance of individual animals through immunization, improved nutrition, or by therapy of diseased individuals. The most extreme example of host manipulation is complete depopulation of a host species for disease control. There is an endless variety of ways in which environmental factors may be manipulated to effect disease; the most important of these for management of wildlife diseases are likely to be through management of human activities.

10.7 How far will the program be taken?

The objective in prevention programs is often total absence of disease from an area. There must be continual surveillance to ensure that the disease has not been introduced or become re-established, negating the effort spent in prevention or eradication. In most control programs, the objective is to reduce the occurrence or effect of the disease, rather than to eliminate it completely. Each program of this type should have a clearly defined objective, e.g., to reduce the prevalence of the disease to less than 10%, or to immunize at least 70% of the animals in the population. For this to be done, the prevalence or severity of the disease must be known prior to the onset of any control, and there must be continual surveillance to monitor the effect of the control program. Choice of a suitable end-point for control may involve some type of benefit:cost analysis and there may be a level of disease below which the cost of further control is greater than the benefit received.

In the case of disease eradication, the objective is total elimination of the disease from the area and the program must be continued until that end is accomplished. A potential problem in eradication programs is that when a disease has been reduced to a very low prevalence in the population, it may be extremely difficult to determine if the disease has in fact disappeared (sampling methods and minimum sample size required if one wishes to have confidence in the absence of disease in a population were discussed in Chap. 7). Criteria developed for evaluating the feasibility of eradicating vertebrate pests (Bomford and O'Brien 1995) are relevant in considering eradication of a disease. These (with modification) include that:

- the rate of removal (of the agent) must exceed the rate of increase.
- immigration of the agent must be prevented.
- all reproductive members of the population must be at risk of removal.
- it must be possible to detect the agent or disease at low prevalence (if it cannot be detected at low prevalence it will be impossible to know if eradication is successful).
- benefit/cost analysis favors eradication over control.
- there must be a suitable socio-political environment.

Bomford and O'Brien (1995) indicated that a negative in any of the first three criteria dooms eradication, and a negative in any of the latter three criteria greatly reduces the feasibility of eradication. Availability of effective intervention to interrupt transmission of the agent and practical diagnostic tools with sufficient sensitivity and specificity are essential elements for eradication to succeed (Ottesen et al. 1998). The potential to eradicate a human disease depends upon humans being essential for the life cycle of the agent, the agent having no other vertebrate reservoir, and the agent not amplifying in the environment (Ottesen et al. 1998).

10.8 How will success be measured?

The final factor that must be considered before starting any program is how the effectiveness of the procedure will be measured. Each management program should contain a predetermined method for assessing its efficacy. This seldom has been done in wildlife disease work. For instance, collection and disposal of carcasses has been a standard technique used for many years during outbreaks of avian cholera, botulism, and duck plague in wild waterfowl. Although it seems intuitively correct to remove carcasses and, hence, decrease the amount of infective or toxic material in the area, I am aware of only one study that attempted to determine if carcass removal had a significant effect on the outcome of an outbreak, although the costs may be very substantial. Evelsizer (2002) found that collection of carcasses had no significant effect on the mortality rate of moulting ducks during botulism outbreaks on wetlands in Saskatchewan, although resource agencies had been spending approximately $1 million/year picking up duck carcasses in western Canada. The conflicting reports by Pursglove et al. (1976) and Montgomery et al. (1979) provide an interesting example of the difficulty in assessing the efficacy of a program, when no method was established for doing so in advance of the action. Pursglove et al. (1976) concluded that "*depopulation*" of more than 6,000 American coots resulted in the termination of an avian cholera outbreak, while Montgomery et al. (1979) concluded that the decline in mortality in a similar outbreak (in which no action was taken) was the result of "*thinning of the bird population in the area as a result of both the disease process and spring migration*". A small experimental study of treatment of nematode infection in rodents demonstrates the need for monitoring to determine if the methodology is successful. Ferrari et al. (2004) treated yellow-necked mice with an anthelmintic. When only females were treated, the prevalence of worms in females declined about 10% but there was no change in the prevalence in males. However, when males were treated, the prevalence and the average number of parasite eggs declined significantly in both sexes. In this case, monitoring indicated the most effective form of management.

Monitoring the efficacy of management, as it proceeds, can be very important for improving the method. Continuous monitoring and accurate diagnosis are essential for assessing the efficacy of a program. If several agents produce a similar clinical disease, there may be efficient control of one of the factors, but there may be little evident effect on the overall clinical disease occurrence. In such situations, there may be an unjustified loss of faith in the management procedure because of an inability to see results.

Disease-management programs often involve a combination of methods, and the approach must be sufficiently flexible to allow change as the process proceeds. The prevalence of disease may change, new factors may be introduced or discovered, and economic and political realities may vary. It is often difficult to maintain enthusiasm for a program over an extended period

unless there is clear evidence that it is successful, and even if the program is successful, the reduced visibility of the disease may result in diversion of effort to other apparently more pressing problems. In disease-control programs "*success often breeds failure*", because relaxation of effort in the later stages of a program may lead to recrudescence of an apparently vanquished disease that "*will return with a vengeance* (Gubler 2001)". As in any other scientific endeavor, the rationale, objectives, methods, and results of all activities should be carefully recorded, so that one can benefit from past experience. The chapters that follow will deal with specific techniques for disease management in free-ranging animals, using examples, some of which were successful! As a final thought, it appears that in general, it is much easier to prevent the introduction of a new disease into an area than it is to control or eradicate an established disease. This thought should be uppermost in the mind of every wildlife manager whenever the translocation of animals or other management that might influence the occurrence of disease is contemplated.

10.9 Summary

- Wild animals live in environments modified by humans. Most attempts to manage disease in wild animals are necessary because of, or are undertaken to mitigate, the effects of other human activities.
- Management is done because the presence of disease in wild animals is considered a threat to human or domestic animal health, or less commonly to alleviate negative effects of disease on populations of desirable wild species.
- Disease management can have one of three basic objectives: prevention, control, or eradication. Prevention involves precluding the occurrence of disease in animals or populations where it does not already occur. Control involves reducing the frequency of occurrence or the severity of existing disease. Eradication involves total extirpation of a disease from an area or population.
- Management may be attempted by manipulating the agent (risk factor), the host population, the environment (including human activities), or by combinations of these methods. A detailed knowledge of the ecology of the disease is required in choosing the most appropriate method.
- Every disease-management program must have a clear rationale, objective and plan of action, as well as a predetermined method for assessing its efficacy.

11 Management of the causative agent/factor or its vector

The most direct method of managing a disease is to eliminate its cause. The basic requirements for management through affecting the agent directly are: (i) a knowledge of the cause, and (ii) some method for its reduction or elimination. The simplest method of eliminating a causative factor is to prevent the introduction of new disease agents into areas where they currently do not exist. In practice, this form of disease prevention usually involves restricting or modifying human activities, rather than physically barring entry of the agent, and it will be discussed in Chap. 15, together with other methods based on influencing human activities.

The term eradication is often used in disease-management programs directed against the agent and this word must be defined carefully because it has been used in many different ways (Yekutiel 1980). In most cases, when eradication is considered, what is intended is elimination of the agent from a defined area, rather than its total extinction. Often, all that is required for effective disease management is reduction of the agent to a level at which its effects become negligible or at least tolerable. It is incorrect to describe such a program as a disease eradication effort and we should more correctly speak of disease control. The discussion that follows will deal with both infectious and non-infectious diseases. While it is dangerous to generalize, non-infectious diseases, such as those resulting from various poisons and toxins, often are technically easier to control than are diseases caused by infectious agents. The major difference between the two groups is the property of reproduction by infectious agents. If a quantity of a toxic material is released into the environment, it will eventually disappear within some finite time, although this period may be extremely long in the case of persistent agents, such as the polychlorinated biphenyls. In contrast, if a finite amount of a new virus or other infectious agent is introduced into a population, the agent may increase in amount and persist indefinitely through continuous replication. In the case of a toxin, management can be directed primarily at preventing the release of additional material into the environment; in the knowledge that the amount already present will disappear spontaneously over time. The disappearance process might be accelerated by management that physically removes the

material from the environment. When dealing with infectious agents, one must be concerned not only with preventing entry of new material into the area, but also with destroying that which is already present at a rate faster than it can be replaced through replication. Because much of the reproduction of many infectious agents occurs in species other than the vertebrate “target” hosts, I have chosen to consider management of invertebrates that serve as vectors, transport hosts and intermediate hosts here, rather than with other aspects of the environment in Chap. 14.

11.1 Elimination of the cause of non-infectious diseases

In this section, I will discuss the management of several diseases that result from human manipulation of poisonous materials. I chose these examples because this is the most immediate type of non-infectious problem for wild animals and because examples are available of programs that have been highly effective, and of others that have been less so. As noted earlier, management of this type of disease generally consists of arresting or reducing the release of some material into the environment. The difficulties encountered are usually related to problems in convincing the public, regulatory officials, and politicians of the need for a control program. Unfortunately, many poisons that have become a problem for wildlife are either highly useful for some function in our society or are a by-product of some useful process. Thus, suspension of their use or emission may entail an economic cost to some group or to society in general. Discussion of the strategies and politics that have been used by those on both sides of various arguments about environmental contaminants is beyond the scope of this book. The traditional role of the disease specialist in such controversies has been to collect factual information on the effects of the compound in question and then to present this material in an objective manner. Such objectivity is still important, however, scientists may have to become more personally and actively involved in some controversies if the problems are to be considered seriously and resolved.

It is easier to demonstrate the need for management of an environmental contaminant that produces acute, distinctive disease that can be linked directly to the compound than it is for compounds whose effects are subtle, prolonged or delayed. Similarly, it is easier to convince people of the desirability of controlling contaminants that have the potential to cause disease in humans than it is for compounds whose effects are confined to wild animals. For example, the response to the discovery of aquatic mercury pollution in North America was almost instantaneous compared to the response to widespread pollution with certain pesticides such as DDT. This is understandable because many humans died or were damaged in ways more horrible than death as a result of aquatic mercury poisoning in Japan (Study group of Minamata Disease 1968). The relationship between the release of mercury into water and subsequent human disease was clearly evident. In contrast, concerns

about the impact of DDT on human health were related to possible long-term, delayed, carcinogenic, or mutagenic effects, and there was considerable controversy as to whether this compound was harmful. Hence, there was not the same urgency to deal with DDT. At present, similar discussion occurs about the toxicity and risks associated with endocrine disrupting compounds (Cooke et al. 2002). The examples chosen for discussion have included some attempts to assess the effectiveness of the management program, a feature that is missing from many other types of disease-management activity.

11.1.1 Mercury in Swedish wildlife

Mercury pollution is not unique to Sweden, but the situation in Swedish wildlife was documented more thoroughly than elsewhere and Sweden was the first country to institute effective management. Mercury poisoning was first diagnosed in a rook examined at the National Veterinary Institute in 1950. By the late 1950s and early 1960s, the occurrence of extensive mortality of seed-eating and raptorial birds had been well established (Borg et al. 1969). Analysis of feathers from contemporary birds and from museum specimens collected between 1815 and 1965 revealed that the mercury content of both terrestrial and aquatic birds had increased during this period (Berg et al. 1966; Johnels and Westermark 1968). The level of mercury in feathers of pheasants, partridge, and terrestrial raptors remained at a low level until about 1940. There was a sudden 10 to 20-fold increase in the level of mercury in feathers of birds collected after 1940 that coincided with introduction of alkyl mercury compounds as seed-dressing agents to control fungal diseases in crops. Observation of this temporal association between occurrence of disease in birds and use of seed-dressing agents was strengthened by documentation of seasonal variation in the proportion of birds with very high levels of mercury in their tissue that coincided with spring and autumn seeding with mercury-treated seed (Borg et al. 1969).

The situation in aquatic birds such as osprey, grebes, and eagles was different in that an increase in the level of mercury in feathers of museum specimens of these species began earlier and was more gradual than that observed in terrestrial birds. This resulted from increased use of mercury as a part of industrialization, with resulting widespread water pollution. However, even in these species there also was a further abrupt increase in the mercury content of feathers in the post-1940 period. This coincided with use of mercury compounds in the pulp and paper industry, and the release of large amounts of mercury into water. Osprey and grebes living on waters contaminated from pulp and paper mills had mercury levels three times those in birds from uncontaminated areas (Larsson 1970). Thus, in aquatic birds there was evidence of both diffuse and point source contamination.

The problem of mercury pollution was largely ignored for some years despite "*urgent and repeated warnings from the National Veterinary Institute during the late 1950s*" which "*were strongly criticized as unjustified*"

(Borg 1977). However, as a result of the persistence of the scientists in explaining the problem, the use of alkyl mercury seed dressings was reduced in 1965 and then banned in 1966 (Larsson 1970). This had immediate and dramatic effects on terrestrial wild birds. In 1964 (prior to the ban) liver from 46.1 and 30.5% of wood pigeons collected by shooting contained >2 and >5 ppm mercury, respectively. In 1966, only 6.4% of pigeons collected had mercury residues >2 ppm and none had residues >5 ppm (Wanntorp et al. 1967). Feathers of juvenile harriers collected in 1965 contained about 16 ppm mercury on average, whereas a sample collected in 1966 contained about 6 ppm. During the period from 1969 to 1976, the levels were 1–2 ppm, similar to museum specimens collected during the 1840–1940 period (Odsjo and Sondell 1977).

Use of mercury in the pulp and paper industry was reduced in 1966 and prohibited in 1967. The effect of reduction of this source of mercury on wildlife was much less dramatic than occurred in the terrestrial environment. This reflects the difficulty in controlling a toxin that may enter the aquatic system from a great variety of sources and also that: "*mercury is very slowly eliminated from the water environment*" (Larsson 1970). Aquatic mercury contamination continues to be a global problem that has not been prevented (Evers et al. 2003).

11.1.2 Lead poisoning of birds

Lead poisoning of waterfowl was recognized in North America as early as 1874 (Phillips and Lincoln 1930) and the cause (the ingestion of spent shotgun pellets) and nature of the disease were described by Wetmore (1919). Research since then, while adding to an understanding of the disease, has not changed the basic facts that birds become poisoned by consuming lead pellets and that the risk of poisoning is related directly to the number of pellets in an area. Lead poisoning of birds occurs from other sources such as from paint peeling from buildings (Sileo and Fefer 1987) and contaminated sediment (Beyer et al. 2000) but these are local problems. A variety of techniques, such as hazing birds away from areas of heavy shot deposition and cultivation to bury shot in the soil, have been used to reduce the extent of poisoning but so long as lead shot are deposited where waterfowl feed the problem will continue. However, "*control, unlike other wildlife diseases, is simple, straightforward, and highly effective – that is, stop discharging lead shot into waterfowl habitat*" (Anderson 1982). Non-toxic shot, effective for hunting waterfowl, has been available for decades and its use has been required by law in some areas for many years but lead poisoning continues in areas where lead shot is used (Nakade et al. 2005). The struggle to ban the use of lead shot has been hampered by intransigent groups who refuse to accept that lead poisoning is a problem. They often ask: "*If lead is such a big problem, where are the bodies?*" One difficulty in convincing this group of the serious nature of the problem is related to the nature of the disease. Although lead poisoning

can result in highly visible die-offs (Amundsen and Eveland 1986), this is the exception and the course of disease in individual birds generally is prolonged, with poisoned birds seeking seclusion by crawling into cover and effectively disappearing. Continual mortality of large numbers of birds can pass unnoticed unless a special effort is made to search for them. For instance, 1,171 sick, dying or dead waterfowl (of which 67% had lead poisoning) were collected on two lakes in Louisiana over a 3.5-month period. During this period, no unusual waterfowl mortality was recognized or reported by hunters in the area (Zwank et al. 1985). Sublethal effects of lead poisoning, such as effects on immune function (Trust et al. 1990; Rocke and Samuel 1991) are even more difficult to detect than overt poisoning.

Although lead shot persist in wetland soil for an extended period, the shot become less available to waterfowl over time. The time period during which shot remain accessible to birds may be very prolonged in some marshes (Oates 1989). However, Humberg and Babcock (1982) demonstrated a significant decline in the rate of ingestion of lead shot by ducks and in mortality caused by lead poisoning during the first year of a partial conversion to non-toxic shot. An estimated 1.4 million ducks were "*spared from fatal lead poisoning*" in 1997, 6 years after a ban on the use of lead shot in the USA (Anderson et al. 2000). Thus, an effective management technique to reduce lead poisoning of waterfowl is available for use wherever those in power can be convinced to institute it. Lead poisoning from shot continues to be a problem in upland birds, such as mourning doves, and there have been calls for use of non-toxic shot for hunting these species (Schulz et al. 2002).

Lead poisoning was the major mortality factor for mute swans in England (Birkhead and Perrins 1985). The source of lead was lead weights used by anglers. The effects of lead poisoning on the population were well documented (Birkhead and Perrins 1985; Ogilvie 1986) and a ban on the use of lead weights for fishing was instituted in the United Kingdom in 1987. Lead poisoning from ingested fishing weights also occurs in common loons (Daoust et al. 1998; Stone and Okoniewski 2001) and the use of lead sinkers has been banned in areas of Canada and the USA (Scheuhammer et al. 2003).

11.1.3 Pesticides

Certain organochlorine insecticides have been recognized as important toxins for birds for many years. The use of some of the worst offenders has been suspended in developed countries, although illegal use still occurs occasionally and some of the chemicals continue to be used in countries without such regulations. Sufficient time has elapsed since the suspension of use of some of these insecticides so that the effectiveness of this management method can be assessed. Three examples will be discussed and, while they are historical, they confirm that "*if remedial action is taken, and remnant populations still persist, such populations can and do respond by recovery*" (Newton 1998).

The history of the use of heptachlor as a seed treatment for wheat in Oregon and Washington is similar to that of mercurial seed-dressings in Sweden. Mortality of pheasants, quail, and magpies was recognized in 1976. By 1977, mortality of adults as well as lowered reproductive success was apparent in Canada geese. The resident population of geese in one area was documented to be declining. This was linked to consumption of treated grain and a recommendation was made that a less toxic insecticide, lindane, be substituted for heptachlor (Blus et al. 1979). Lindane was substituted for heptachlor in part of the affected area in 1979 and throughout most of the area in 1981. Reproductive success of the geese improved dramatically after the suspension of heptachlor use. Nest success was 52% in 1978 and 84% in 1980; hatching success was 50% in 1978 and 81% in 1980 (Blus et al. 1984). Mortality of adults declined and the nesting population increased. In 1978, the average content of heptachlor epoxide in goose eggs was 2.93 mg/g; the highest level found in any egg in 1981 was 0.47 mg/g and some eggs had no detectable heptachlor epoxide. No adverse effects of lindane were detected (Blus et al. 1984).

Newton et al. (1992) described the decline of populations of British sparrowhawks and kestrels as a result of the widespread use of the pesticides aldrin and dieldrin and the subsequent recovery of these populations following cessation of use of these compounds. In both of the heptachlor and the aldrin/dieldrin instances, the management technique of preventing introduction of the causative factor appears to have been totally successful.

The effects of suspension of use of some other pesticides have been much less dramatic. DDT was utilized throughout the world and DDE, a toxic metabolite of DDT, was usually "*the most common organochlorine pollutant found in wild birds*" (Ohlendorf 1981). While DDT was not highly toxic to birds, DDE was a major cause of eggshell thinning, resulting in reproductive failure in many species of birds. Use of DDT declined in the USA during the 1960s (Spitzer et al. 1978) and registration, except for very limited uses, was cancelled in 1972. Most industrialized countries instituted similar bans, but manufacture and use continues, although at a reduced rate, in some parts of the world (Chen and Rogan 2003), so this pesticide is of more than historic interest. DDE is very persistent, both in the environment and in tissues, whereas DDT generally does not persist in the environment for more than 2 years (Anderson et al. 1984). The level of these compounds has declined in birds, although the reduction following cessation of use was not as dramatic, nor as complete, as that which occurred after suspension of use of heptachlor. Spitzer et al. (1978) observed a decline in residues of DDE in osprey eggs over the period from 1969 to 1976, which coincided with an increase in nesting success. Morrison et al. (1978) found a –93.6% change in the level of DDE in eggs of olivaceous cormorants from 1970 to 1977. Spraying of DDT on a university campus between 1955 and 1962 was associated with both direct mortality and a population decline of robins. Die-offs continued until 1965, but mortality caused by DDT ceased by 1969 and reproduction returned to a "*pre-DDT*" level by 1979 (Beaver 1980). Chapdelaine et al. (1987) described

a marked improvement in breeding performance of northern gannets that coincided with a decline of DDT and dieldrin residues in eggs.

Not all studies revealed such optimistic trends, e.g., Ohlendorf (1981) reported that levels of DDE in black ducks and mallards in the Atlantic Flyway declined significantly from 1969–70 to 1972–73, but then remained unchanged from 1972 to 1976. Eggs of brown pelicans collected during 1975–1981 had about one-half the level of DDE of eggs collected in 1970, but that levels did not decline from 1975 to 1981 (King et al. 1985).

Efforts to control DDT and its breakdown products were hampered not only by the large amount of these compounds in the environment and their persistent nature, but also by the inability to prevent the continued addition of new material. DDT continued to be manufactured and used in countries that are the wintering area for North American birds, and the chemical was used illegally in the USA for many years (Anderson et al. 1984; Ohlendorf and Miller 1984; King et al. 1985). Another source was contamination with DDT in related pesticides, e.g., more than 234,000 kg of the compound dicofol, that contains 6–9% DDT and DDE was used in California in 1981 (Ohlendorf and Miller 1984). As noted earlier, it is hard to convince the public that a problem exists if the effects of a contaminant are difficult to demonstrate in a convincing manner. The effects of DDT on birds were insidious; it seldom killed birds directly, and the evidence of a causal link to reproductive failure was "*entirely correlational*" for a time, "*a point that was exploited to the full*" by those interested in retaining use of the pesticide (Newton 1998).

These examples illustrate that some non-infectious causes of disease can be managed effectively but that public and political will to effect the change is often difficult to rally, unless it is possible to present clear, incontrovertible proof of direct disease effects.

11.2 Attacking the cause of infectious diseases

The potential success of any management program for infectious disease is influenced by many factors, not least of which is the nature of the causative agent. In discussing eradication of infectious diseases in humans, Yekutiel (1980) divided agents into two general groups. **Endogenous** agents are those that are often present in the body without causing obvious disease, or that are ubiquitous healthy survivors in the external environment. They produce disease only under special circumstances such as when the host's resistance is impaired or when there is some unusual route or intensity of exposure. Diseases produced by these agents include infections of the urinary, respiratory, alimentary, and female genital tracts, wound infections and, on occasion, septicemia. The terms opportunistic or facultative pathogen are applied to this type of agent and the group includes bacteria such as *Escherichia coli*, *Arcanobacterium pyogenes* and *Fusobacterium necrophorum*, and the fungus *Aspergillus fumigatus*.

Exogenous agents are not present in healthy animals, but are acquired from outside sources, often from other animals. They produce well-defined disease within a predictable and usually short time after introduction to the body and most do not survive for extended periods free in the external environment. These agents usually do not form commensal or persistent chronic infections, although there are many exceptions, such as tuberculosis, to the latter. Examples in wild animals include rabies and canine distemper viruses and *Francisella tularensis*, the bacterium that causes tularemia. There is an intermediate group of agents, such as the Salmonellae and *Mannheimia haemolytica*, which depending on the circumstances, might behave as either endogenous or exogenous agents. Yekutiel concluded that eradication, even on a local basis, was possible only in human populations for infectious diseases of the strictly exogenous type.

The concept of endogenous/exogenous agents is useful for developing a general principle that the more potential sources exist for an agent, the more difficult it will be to reduce or eliminate the disease through attacking the agent. Consider, for example, that one wished to control a disease that caused mortality among caribou calves in Greenland (Thing and Clausen 1980). *Escherichia coli*, serotype O55, was the only pathogen isolated from sick calves with diarrhea and arthritis during a year when the mortality rate among calves was estimated at 50%. The same serotype of *E. coli* was isolated from feces of normal adult caribou in the area and has been found in many other species, including humans, indicating that this is clearly an endogenous agent. In this instance, it would be impractical or impossible to eliminate the agent. The basic problem in this situation was poor range condition, resulting in malnourished cows that produced weak calves vulnerable to an opportunistic agent (Thing and Clausen 1980). Disease management should probably be directed at the habitat and the caribou population rather than directly at *E. coli*.

In contrast, if one was confronted with an agent that had only one vertebrate host, (hence the potential source is limited), or whose transmission is dependent on some identifiable part of the external environment, one might consider a more direct attack on the agent. There are three basic points at which a disease agent could be attacked. The agent might be attacked when it is: (i) within the host, (ii) free in the environment, or (iii) in some other carrier, reservoir or vector species. These will be discussed in turn, beginning with methods used to attack the agent when it is within the host.

11.2.1 Attacking the agent within the host

A commonly used method to manage disease in humans and domestic animals is destruction of the agent within the host through use of some drug, such as an anthelmintic or antibiotic. This method has been applied to a limited degree in wild animals. Anthelmintics were used to control *Protostrongylus* spp. lungworms in certain populations of bighorn sheep, where it was impossible

to disperse the animals from heavily contaminated areas or to control the snail intermediate hosts (Schmidt et al. 1979). Sheep were lured to a bait station and preconditioned to eating bait, to which anthelmintic was then added. Treatment was timed to kill larvae present in pregnant ewes and, hence, to reduce transplacental transfer of infection to lambs in utero. Treatment also reduced the number of adult worms carried by the sheep and environmental contamination with larvae. In some herds, the survival rate of lambs from treated ewes was of the order of 70–80%, whereas only 5% of lambs from untreated ewes survived (Schmidt et al. 1979). Goldstein et al. (2005) added anthelmintic to free-choice mineral mix to treat lungworm infection in bighorn sheep. It was thought that high levels of parasitism were increasing stress on ewes and, hence, reducing lamb survival. Treatment reduced output of larvae in the feces of treated sheep but there was no effect on fecal glucocorticoid metabolites (a measure of stress) or on lamb survival. To achieve long-term reduction in number of lungworms, treatment had to be administered repeatedly at 4–6 week intervals. Some sheep did not consume adequate anthelmintic to eliminate the parasites.

There have been a number of trials to test the feasibility of reducing risk to humans from the tapeworm *Echinococcus multilocularis* by administering the anthelmintic praziquantel in baits to kill the adult tapeworm in the intestine of wild foxes (Schelling et al. 1997; Tackmann et al. 2001; Hegglin et al. 2003; Ito et al. 2003). Treatment has been effective in substantially reducing but not eliminating the infection rate in foxes. Baiting had to be repeated at about 4 to 6-week intervals to control re-infection and the prevalence in foxes rebounded after treatment ceased (Ito et al. 2003). This technique may be practical in an urban situation (Hegglin et al. 2003) but its feasibility for use over larger areas is still unclear.

Drugs have been used experimentally to reduce infection rates of parasites in red grouse (Hudson 1986), Soay sheep (Gulland et al. 1993), cliff swallows (Brown et al. 1995), snowshoe hares (Murray et al. 1997), reindeer (Albon et al. 2002), pheasants (Hoodless et al. 2003), and mountain hares (Newey and Thirgood 2004). In these trials, drugs were administered directly to individual animals and the intent was to reduce parasite numbers rather than to eradicate the parasite. Control was achieved on a local, short-term basis. This type of local treatment of individual animals only is practical for situations where intensive management can be applied. This situation may become more frequent in future for intensively managed populations on small areas, such as wild boar on small hunting grounds (Rajkovic-Janje et al. 2004). Anthelmintic treatment was continued over a 7-year period to counteract "*extremely heavy nematode*" infections among roe deer (Duwel 1987), resulting in a 95% reduction in mean worm burden, a doubling of fawn body weight and an improvement in antler size, in comparison to the situation prior to treatment. Anthelmintic treatment for the fluke *Fascioloides magna* was considered to be "*practical and logical*" for white-tailed deer in Texas that are managed intensively and given supplementary feed (Qureshi et al. 1990).

At this time, it appears that drugs could be used to attack some disease agents while they are in wild animals, but the major problem lies in the mass delivery of drugs to wild populations. Other potential problems are illustrated in the history of this type of program in management of two infectious diseases of humans. The campaign to control malaria, which is a risk to approximately one-third of the world's human population and causes 1–3 million death annually, demonstrates problems that may arise when chemical control of a disease agent is applied widely and for an extended period of time. Development of the drug chloroquine during World War II was an important milestone in the anti-malarial campaign. The drug was so successful initially that global eradication of malaria was considered a possibility. Massive programs were designed to deliver the drug; however, chloroquine-resistant malaria was soon recognized and is present in malaria-endemic areas throughout the world (Harrus and Baneth 2005; Shanks et al. 2005). New drugs were introduced but malaria resistant to multiple drugs developed. Control of malaria has been complicated further by emergence of insecticide-resistant mosquitoes that act as vectors of the disease. Alternatives to chemotherapy, that have been considered, include a return to environmental management for mosquito control, development of vaccines, and even genetic manipulation of mosquito populations (Collins et al. 1986).

Use of mass medication to control schistosomiasis, another major infectious disease of humans, has had a shorter history than that of malaria. Selective population chemotherapy has been used in which either only active cases of infection are treated, or treatment is directed at a particular group within the population, e.g., children, when the prevalence reaches a certain level. This selective approach requires adequate surveillance and a reliable diagnostic method. Jordan and Webbe (1982) reported that, if population participation in the program was good, a rapid fall in prevalence of infection should be expected after treatment. The degree of transmission control attained depended on the extent of the reservoir of infection remaining in the community after treatment. The size of this reservoir was dependent on the cooperation of the population in being examined and treated, the sensitivity of the detection method used, the efficacy of the treatment, and the extent of immigration of infected individuals into the area. These same factors would apply in any attempt to control a disease in wild animals by treating infected individuals.

The objective of a schistosomiasis control program in Brazil was to reduce prevalence of infection in the population to <4%. The method used depended on the prevalence, measured by examining children at 6-month intervals. Where prevalence was >20% in 7 to 14-year-old children, everyone in the population was treated. Where prevalence was 4–20%, only persons 5–25 years of age were treated, and where or when prevalence was <4%, only infected children were treated (Jordan and Webbe 1982). In sub-Saharan Africa the objective of schistosomiasis control is to treat ≥75% of school age children in areas with a high burden of schistosomiasis by 2010. Problems

have been encountered in some areas in getting sufficient compliance from the population and because of immigration of infected people (Southgate et al. 2005). The type of detailed surveillance necessary for such programs is impossible in most wild species.

Although acquired resistance to drugs has not yet been a major problem in schistosomiasis control (Botros et al. 2005), this is a definite risk in any program dependent upon continued and widespread use of chemotherapy, because of the selective pressure for resistant organisms. While selection for resistance often is thought to require lengthy exposure to a drug, antibiotic-resistant staphylococci developed and persisted for 4 years in humans after treatment for 1 week (Sjölund et al. 2005). One method of reducing development of resistance is through use of combinations of drugs or rotational use of different drugs (Harrus and Baneth 2005) but this carries the risk of building multiple drug resistance (Mas-Coma et al. 2005).

11.2.2 Attacking the agent in the external environment

If the disease agent is concentrated in some location outside the host, it may be possible to exert management there and avoid infection and injury of the host (a major disadvantage of any disease-management program based on attacking the agent within the host is that the agent may already have caused significant injury to the host before the control is effective). The rationale for collection and disposal of carcasses during disease outbreaks is to eliminate agents contained within the carcass as a source of infection for other animals. Friend and Franson (1999) provide detailed recommendations for carcass collection and disposal. These were developed for use in outbreaks in birds, but they are readily adapted to other circumstances. Other examples of this type of control include the disinfection of soil to destroy parasite eggs (Skrjabin 1970) and *Histoplasma capsulatum* (Weeks 1984); disinfection of water to control agents, such as those causing duck plague (Pearson 1973), avian cholera (Gershman et al. 1964) and anthrax (Pienaar 1967; Government of the Northwest Territories 1999), and decontamination of raccoon latrines to destroy eggs of the nematode *Baylisascaris procyonis* (Page et al. 1999). The efficacy of such measures seldom has been tested adequately. Rosen and Bischoff (1949) were unable to recover *Pasteurella multocida* from pond water after treatment with copper sulfate but no untreated ponds were examined for comparison. Use of lime to destroy helminth eggs about artificial feeding sites for 3 years eliminated *Protostrongylus* spp. lungworms from hares on an isolated island, while hares on the adjacent untreated mainland continued to suffer from severe protostrongylosis (Skrjabin 1970). However, this was an unusual situation in which all of the hares were concentrated by artificial feeding in the winter, and only the immediate area about the small feeding sites required treatment. Carcass collection and disposal seems intuitively correct and may be a visible activity when disease management is

demanded but we found that only about one-third of duck carcasses were collected during carcass cleanups in a botulism outbreak (Cliplef and Wobeser 1993). There was no significant difference in mortality of radio-marked mallards, as a result of botulism, on wetlands where carcass collection was done and on similar-sized wetlands with no carcass collection (Evelsizer 2002).

Occasionally, it may be possible to reduce or eliminate an agent at large over an extended area. The North American screwworm *Callitroga hominivorax* will be used as an example. While the program for its management was not done to reduce disease in wild species, eradication had important implications for wild deer. This fly is an obligate parasite that must deposit its eggs into a wound on a homeotherm; the resulting larvae, or screwworms feed on living tissue and provide a portal of entry for further larvae or other pathogens. As with many other diseases, this parasite only became a problem as a result of habitat disruption. Expansion of cattle production, together with habitat changes that favored woody legumes used as food by the adult flies, allowed the fly population to expand greatly in the southwestern USA. The parasite was transported to Florida with infected cattle and became established there (Richardson et al. 1982). In one area of Texas, 80% of deer fawns died in years of severe screwworm infestation, compared to 25% in years of light infestation (Strickland et al. 1981).

The female fly breeds only once each year and management took advantage of this by releasing massive numbers of artificially reared, irradiated male flies that were sterile but sexually active. This 'sterile insect technique' (SIT) was used first on the islands of Sanibel and Curacao, and then in Florida, resulting in eradication of the fly. The program in Florida during 1958 and 1959 cost $10 million and involved the release of up to 3,500 sterile flies/km^2 in some areas (Richardson et al. 1982). The program was extended to Texas and other areas of the southwestern USA where the intent was to prevent annual incursions from an enzootic area in Mexico. Sterile flies were dropped from airplanes in a broad band along the border with Mexico. This resulted in suppression of the problem for a number of years; however, there were several outbreaks despite the control program. These were attributed to genetic diversity in the wild flies, some of which were different from the artificially reared sterile flies (Richardson et al. 1982). Control of screwworm was followed by a rapid expansion of deer populations (Strickland et al. 1981). This created new problems for the wildlife manager in the form of increased crop depredation (Richardson et al. 1982) and concern that the expanded deer herd might represent a risk to livestock from other diseases, such as piroplasmosis (Marshall et al. 1963). Subsequent use of SIT resulted in eradication of screwworm from Mexico and Central America and reinvasion has been prevented by continued release of sterile flies in a narrow band in Panama (Benedict and Robinson 2003).

SIT was used successfully to eradicate screwworm from North Africa (Lindquist et al. 1992), as well as a tsetse fly from a large island in Zanzibar (Vreysen et al. 2000) and a fruit fly from Japanese islands (Koyama et al. 2004).

In some of these situations, other methods were used to reduce the insect density prior to release of sterile insects. Genetically modified insects that are sterile might be used in future in place of radiation-sterilized males (Benedict and Robinson 2003).

11.3 Management of invertebrates involved in disease transmission

Invertebrates are involved in a variety of ways in the transmission of infectious agents among vertebrates. The simplest form is when the invertebrate carries a disease agent on its mouthparts or body from one vertebrate to another. The invertebrate is sometimes called a mechanical carrier or, in the case of a biting insect, a flying pin. I prefer the term "transport host". The infectious agent usually remains viable on the invertebrate for only a short period of time but the invertebrate extends the distance over which contact occurs between infected and uninfected vertebrates. Examples of this form of transmission include the spread by mosquitoes of poxvirus among wild birds and transmission of anthrax by blowflies (Braack and de Vos 1990). This form of transmission is a facultative association in that the disease can be transmitted among vertebrates by other routes and is not dependent on the invertebrate.

The term vector is used here in a restricted sense for "*an invertebrate that transmits an infectious agent among vertebrates and in which the agent multiplies or completes some required portion of its life cycle*" (Wobeser 2006). The vector serves as a site for multiplication and/or development of the agent and may extend the possibility for transmission in both space and time. For instance, the agent may persist for an extended time, such as over winter, in the invertebrate, without access to a vertebrate host. Examples of various types of vector relationship include plague, in which the causative bacterium *Yersinia pestis* multiplies within the flea vector; the meningeal worm *Parelaphostrongylus tenuis* in which the larvae undergo development in a snail intermediate host; and *Leucocytozoon simondi*, a protozoan parasite of waterfowl, in which both multiplication and development occur in the blackfly vector. The vector may be either a facultative or an obligate part of the ecology of a disease. For example, the bacterium *Francisella tularensis* may be transmitted by a variety of methods, one of which is by arthropod vectors, whereas the only way that a deer can become infected with *P. tenuis* is through consumption of an infected snail.

Vector-borne diseases are extremely important in human and veterinary medicine, and manipulation of vectors has been a major method for the management of a number of diseases of these species. The general strategy has been to either reduce the risk of exposure of the vertebrate host to the vector, (i.e., to block transmission from the vector to the vertebrate) or to reduce the

general level of environmental contamination with the disease agent by attacking the vector. The most common method used has been to reduce the population of vectors. Some general features about vectors are important for consideration in such a program:

- the prevalence of infection in the vector is usually very low compared to that in the vertebrate host.
- the expected life-span of most vectors is short and, in many arthropod-borne diseases, the incubation period of the disease is similar to that of the life expectancy of the vector (Anderson 1981) (in general, tick vectors live longer than insect vectors).
- vector species often have an ability for rapid replication, or a high rate of natural increase.
- vector species responsible for disease transmission often comprise only a small proportion of the total population of closely-related species in an area, e.g., *Leucocytozoon simondi* is transmitted by only a few among the many species of blackfly present in an area.
- the activity of many vectors is highly seasonal and affected markedly by climate and weather (the geographic distribution of vectors is often delimited by climate).

Taken together, these factors indicate that invertebrates responsible for transmission of a disease may represent only a very small proportion of the total population of similar animals in the environment that will be effected adversely by the control program, that they may be active and available for certain types of control only during a restricted time period, that the majority of individuals of the vector species will not be infected with the disease agent, and that the population will likely have a very high turnover rate and intrinsic ability to rebound after population reduction.
General methods used for management of invertebrates include:

- reduction or elimination of habitat required by the invertebrate. This often involves changes in aquatic environments required by larval insects or landscape management on small areas to reduce ticks (Ginsberg and Stafford 2005).
- reducing or preventing exposure of vertebrates to infected invertebrates.
- Increasing the mortality rate of the invertebrates with chemicals.
- Increasing the mortality rate of the invertebrates using biological control agents.
- Impairing invertebrate reproduction using SIT.

The association between swamps and mosquito-borne diseases of humans has been known for centuries and draining wetlands was highly successful in reducing or eliminating malaria and yellow fever endemic areas (Gubler 2001). However, the value of wetlands for other purposes largely precludes

this method for managing diseases of wild animals. The reverse side of this situation is that human-induced habitat changes, including deforestation, agricultural development, and alterations in water storage and irrigation have created suitable environments for many invertebrates that transmit diseases (Jardine et al. 2004; Lindsay and Birley 2004; Harrus and Baneth 2005). Some of these adverse effects might be managed through public education and advance planning to reduce suitable habitat for vectors. Although simple means such as window screens, bed nets, and self-protection measures are effective in humans, there are no feasible methods for preventing contact between invertebrates and vertebrates in wild populations.

"*With the advent of inexpensive, quick-acting, and long-residue insecticides during the 1940s, vector species could be controlled on a scale never approached before. The successes achieved, especially in the field of animal diseases, were spectacular indeed*" (Simons 1981). The initial success of the "*quick fix*" or "*magic bullet*" approach (Gubler 2001) led to loss of interest in other control methods, such as environmental modification for the control of mosquito vectors of malaria. However, problems rapidly became evident with the widespread use of chemical pesticides. Many of the chemicals used for invertebrate control (insecticides, acaricides, and molluscicides) are broad-spectrum poisons with serious, undesirable side-effects. This problem is so well known that it will not be described here, other than to mention two examples of unexpected side-effects of chemicals on disease. Because the chemicals are not specific in their toxicity, invertebrate predators of the harmful invertebrate species may be killed by the chemical. MacDonald (1972) cited examples in which a vector population increased markedly in the season after treatment, supposedly as a result of "*release*" from the control normally exerted by their predators. MacDonald (1972) also cited instances in which control of the vector of one disease resulted in an entirely new disease problem. Insecticide use for control of mosquito-borne malaria in Bolivia was thought to have resulted in widespread poisoning and mortality of urban cats. This was followed by invasion of homes by wild rodents that carried the virus of Bolivian hemorrhagic fever and by an outbreak of that disease among humans. Pesticides with very low vertebrate toxicity, such as some biological control agents, may deplete the other invertebrates that are important as food for wildlife (Hershey et al. 1998; Pinkney et al. 2000).

Many invertebrates rapidly become resistant to chemical agents. Development of acquired resistance is directly related to the success of control programs, with invertebrates being rigorously selected for various adaptations to the new toxic environment. This is a global problem and as early as 1972 >100 arthropods involved in transmission of diseases of humans or animals were resistant to at least one insecticide (MacDonald 1972). The development of resistance was somewhat erratic and a compound might be effective in one area and totally ineffective in another. This was evident in the use of insecticides for the control of the flea vectors of plague. DDT was efficacious for the control of fleas on voles in California in 1966 (Kartman and Hudson 1971)

while massive treatment (10,000 kg in one area during 3 months) failed to control fleas on rats in Vietnam at about the same time, because the fleas were resistant to the chemical (Cavanaugh et al. 1972). Similarly, carbaryl was effective in controlling fleas on a variety of animals in Colorado (Barnes et al. 1972, 1974), but failed in California because of resistance (Barnes 1982). Although resistant individuals have been found in areas where a chemical has never been used, the development of resistance generally followed widespread use of the particular compound in an area. The greater the selective pressure exerted through use of the chemical, the more rapidly resistance developed (Crow 1957). Mechanisms involved in the evolution of resistance to organophosphate insecticides in mosquitoes have been described by Raymond et al. (2001).

Various insecticides have been used to reduce flea populations on rodents in areas where plague occurs. Insecticide may be applied by dusting the rodent's burrow, or with the use of bait boxes containing insecticide. The use of insecticide may be designed to reduce the risk to humans at campgrounds and parks (Mian et al. 2004) or to reduce mortality in desirable species such the black-tailed prairie dog (Seery et al. 2003). Insecticide was applied to approximately 80,000 prairie dog burrows in Wyoming in order to control an outbreak of plague that threatened one of the only surviving wild populations of the endangered black-footed ferret (E.T. Thorne, personal communication). Efficacy of such treatment has usually been assessed on the basis of the reduction in ectoparasites, although Seery et al. (2003) reported that plague caused high mortality of prairie dogs on some untreated colonies that were near treated colonies in which plague did not occur. Treatment has been reported to halt the spread of plague within some colonies (Hoogland et al. 2004). Karhu and Anderson (2000) found that application of one insecticide caused a decline in some non-target insects in the treated area. Methods of remote delivery of acaricides for local tick control, including bait boxes for rodents and topical delivery systems for deer, have been developed (Ginsberg and Stafford 2005). The systemic antiparasiticide ivermectin has been delivered to deer in grain for tick control, but its widespread use was considered unlikely because of potential residues in meat of deer killed by hunters (Ginsberg and Stafford 2005).

Monath (1984) observed that little real progress had been made in the prevention of many vector-borne diseases and that the geographic range of, as well as morbidity and mortality from some vector-borne viral diseases of humans had increased. This represents the notable failures of the past 30 years described by Gubler (2001). Realization of the danger in relying on chemical control of vectors has given rise to the concept of integrated control, in which use of chemicals is combined with a variety of biological controls and environmental manipulations. Even closely related diseases with common vectors may require totally different control strategies and the specific control program for each disease depends upon understanding the ecology, population dynamics, and specific requirements of the vector. The general strategy is to

use environmental manipulation, and perhaps biological controls, to reduce the abundance of the specific invertebrates that act as dangerous vectors. A major advantage of this type of integrated technique for control of the snail vectors of human schistosomes is that the effect is persistent without continual reapplication (Jordan and Webbe 1982). The disadvantages are that it may be some time before the benefits are apparent, and the procedure may be more demanding to implement than use of a chemical agent.

Although control through use of biologic agents is often cited as a part of integrated control programs, biological agents such as predators or pathogens seldom have been used on an operational scale to control disease vectors to date. In a recent review, Ginsberg and Stafford (2005) concluded that effective biological control of ticks has not been achieved. Biologic control methods are usually directed against the immature stages of the vector and, since a large proportion of these die of natural causes, a control agent would have to "*achieve a very high kill in order to reduce significantly the adult population*" (MacDonald 1972). A potential method of biologic control is through some type of genetic manipulation of the vector. The release of sterile flies, as described for screwworm control, has been attempted on a trial basis for mosquito control, apparently without notable success. MacDonald (1972) reported that attempts to use this method on some species had failed, while limited trials with *Culex* spp. resulted in a reduction in the natural population. Jordan and Webbe (1982) observed that while the method might work for some insects, it would not be applicable to many gastropods, which are hermaphroditic. Other methods, such as development of genetic strains of vector refractory to infection with disease agents (Collins et al. 1986) do not appear to have advanced beyond an experimental stage.

In an integrated control program, chemical agents are used in a selective manner to reduce vector populations in focal areas or when the level of activity or prevalence of the disease agent reaches a threshold limit, above which there is significant risk to humans or animals. This type of precisely targeted use of pesticides obviously requires continual monitoring and surveillance of the level of disease activity. Barnes (1982) stressed the need to avoid the use of "*routine and repetitive treatments which might lead to development of insecticide resistance*". When insecticide was used for plague control, it was applied to burrows or runways for animals that have identifiable burrows or trails, and through the use of insecticide bait stations for species that do not use burrows. General area application of insecticide has been unsuccessful in plague control, except where the amount of insecticide used was so great that it endangered humans and other species (Barnes 1982).

Control of vectors has not been used widely for the control of disease in wild animals, except for those diseases shared with man and livestock, and to benefit endangered species; however, we can gain immensely from the experience gained in the efforts that have been made to control diseases such as trypanosomiasis, malaria and schistosomiasis, so that we do not make the mistakes apparent in those campaigns.

11.4 Summary

- In order to manage disease by attacking its cause, it is necessary to know the cause and to have an effective method of dealing with it.
- It is often technically easier to eliminate the cause of a non-infectious disease than it is to eliminate an infectious agent.
- Elimination of toxic agents usually involves changing human activities. It is easier to convince people of the need for control of acutely toxic substances than it is for toxins that produce subtle or delayed problems, although the latter may be more damaging to wild animals.
- Infectious agents may be divided into two classes. Endogenous agents are present in normal hosts without causing obvious disease or are ubiquitous in nature and produce disease opportunistically. Exogenous agents are not present in normal hosts, produce clearly defined disease when introduced, and do not persist in the environment. It is impossible to eliminate endogenous agents, even on a local basis, because of the many potential sources.
- Infectious agents may be attacked free in the environment, e.g., by disinfection, within carriers, vectors, or animal reservoirs, or within the host (by chemotherapy). A disadvantage of attacking the agent within the host is that substantial injury may have occurred prior to treatment.
- Any program based on widespread and repeated use of chemotherapy or biocides to attack living agents or vectors is likely to stimulate development of acquired resistance in the target organism. Such methods lose their effectiveness rapidly.
- Precisely targeted treatment, based on detailed knowledge of the ecology of the disease, that uses minimal amounts of a variety of chemicals and that is coordinated with other forms of management, is likely to be more effective than mass treatment with a single agent, in the long term.
- Most methods that have been used to attack infectious disease agents in wild animals are of untested efficacy.

12 Disease management through manipulation of the host population

> "*Practical control efforts are inevitably determined by the size, structure, and distribution of the community concerned*"
>
> (Fine et al. 1982)

This chapter deals with management of disease through manipulation of the distribution, size, density, and composition of animal populations. The animals considered are the vertebrates involved in disease, except for humans. I arbitrarily assigned disease-management techniques based on manipulation of invertebrates, such as the snail intermediate hosts of many helminths and the insect vectors of certain viral diseases, as well as the management of human activities, to other chapters. Methods that involve alteration of the resistance of individual animals through immunization and chemotherapy also will be discussed elsewhere. The methods discussed here are based on preventing contact between disease agent and host or on reducing or preventing the transmission of the agent among hosts. The actions have been arranged in a series of steps, with each successive step involving an escalation in intensity of the action and in the degree of violence imposed upon the population. One must expect that each step in this escalation will be met with decreased public acceptance and increased resistance. The latter must not be underemphasized and a program that seems rational, logical, and in the best interests of a wild species in a biologic sense, may be totally unworkable if politicians feel that it is unacceptable to the public. A disease-management program that is tolerated in one area may be totally unacceptable in another, because of variation in the public's attitude toward animals. For example, the use of rodenticides to kill ground squirrels for plague control was acceptable in California where the animals were viewed as agricultural pests, but was unacceptable in other areas where the animals were viewed "*as a normal and attractive part of the biota*" (Barnes 1978). Similarly, sodium fluoroacetate (1080) has been used extensively to poison brushtail possums in New Zealand, while use of this poison is severely restricted or banned in many other countries. No population control action should be taken lightly but, if convinced that the technique is needed, justified and will be efficacious, efforts should be directed at educating the public of its benefits and costs.

12.1 Defining the population(s) of interest

Before beginning any management based on manipulating animals, it is critical to understand the role of various species and other sources in the disease. It makes no sense to try to manage one species if there are other sources of the disease that cannot be controlled. Similarly, it is wasteful to attempt to manage a species that may be infected by an agent but which plays no role in transmission of the agent or in allowing it to persist. Thus, it is important to determine when dealing with multihost diseases whether the various species are maintenance, spillover or dead-end hosts, so that management is directed at those species that are important in disease transmission. As an example, bovine tuberculosis occurred in cattle, water buffalo, and feral pigs in the Northern Territory of Australia. When the population density of cattle and buffalo was reduced from about 20 to $<0.1/km^2$, the prevalence of tuberculosis in pigs dropped from 16 to 0.25% without any direct management directed at pigs (McInerney et al. 1995). Similarly, control of rabies in dogs eliminated rabies from both dogs and wild carnivores, at least for a time, in the Serengeti (Cleaveland and Dye 1995).

It also is important to understand the demographic features of the population that will influence the management. Is the population closed or open? A closed population, in which all increases and decreases are the result of births and deaths, may be much easier to manage than an open population in which recruitment by immigration is important (Merril et al. 2006). Immigration may serve as a source of both infected individuals exposed elsewhere and of susceptible individuals (Fulford et al. 2002). Does the area to be managed have characteristics of a sink or source situation? A sink habitat is one in which local births are insufficient to balance death in the absence of immigration, whilst production exceeds local mortality in a source habitat. If immigration is important, the distance to potential source populations may be important in the rate of recovery. How rapidly can the species recover after disturbance? Populations of *r*-strategists such as most rodents are likely to recover much more rapidly than *K*-strategists. For instance, models predicted that about 65% of foxes, 87% of rabbits, and 97% of house mice in Australia would have to be culled or sterilized annually to stop maximum population growth, as can occur after large population reductions (Hone 1999).

12.2 Manipulation of animal distribution

The general objective of this type of management is to reduce contact between infected and uninfected animals in the case of infectious diseases, or between animals and risk factors in the case of non-infectious diseases. The management may be done to reduce the impact of an ongoing disease or to prevent occurrence of a new disease.

12.2.1 Dispersal

A common recommendation for action in the face of an epizootic, particularly among highly mobile species such as birds, is to disperse the animals away from the immediate area. The underlying assumption is that acquisition of the disease is associated with a particular geographical area. Dispersal techniques have been used in many situations, including moving waterfowl from areas heavily contaminated with lead shot (Anderson 1982; Esslinger and Klimstra 1983), from the site of botulism outbreaks (Parrish and Hunter 1969), and away from an outbreak of duck plague (Pearson 1973). Dispersal has been used to prevent geese using fields sprayed with pesticide (Anonymous 1956) and from feeding on pesticide-treated seed grain (Bailey et al. 1972), and for removing whooping cranes from the area of an avian cholera outbreak (Zinkl et al. 1977). An attempt was made to herd wild bison away from the site of an anthrax epizootic (Novakowski et al. 1963).

The effort required to move or disperse animals from an area must not be underestimated. Meagher (1989) reviewed attempts to displace or move bison in Yellowstone National Park in relation to possible management of brucellosis and concluded that, despite very intense efforts, "*bison can be moved only where they want to go*". Rosen and Bischoff (1953) described the use of grenades and rockets launched from rifles, signal flares fired from Very pistols, an airplane, an airboat, a smoke-generating machine and an unspecified number of personnel to drive ducks from the site of a botulism outbreak. The pyrotechnics used in this operation makes the description of disease control as a battle seem particularly apt!

Many reports of dispersal programs comment on the rapid return or repopulation of the area (Anonymous 1956; Novakowski et al. 1963) unless harassment is continued or the habitat is modified to make it unattractive, such as by draining a wetland to displace waterfowl. Devices for frightening animals and other methods of harassing animals may be ignored by wild animals (VerCauteren et al. 2005) or lose their effectiveness with continuous use. For instance, we found that a propane exploding device (scare cannon) was effective for <1 week in preventing ducks from using a 30-m-long island that was a focus of botulism. Displacement of animals is easier if attractive alternate habitat is available nearby. Such a site may have to be created as part of the displacement technique. Parrish and Hunter (1969) were able to have hunting suspended and new habitat created by flooding on a nearby area, to facilitate "*herding*" ducks from a botulism-prone area. Rosen and Bischoff (1953) established an artificial feeding site nearby to lure ducks away from a botulism outbreak and we have used this method to deal with a botulism outbreak among ducks concentrated at an artificial feeding site.

The efficacy of animal dispersal as a method to reduce mortality in an outbreak has never been tested objectively, although some authors have suggested that it has been effective. For instance, dispersal, together with treatment of sick birds and carcass cleanup, was thought to have "*saved*" 250,000 waterfowl

during a botulism outbreak in California (Parrish and Hunter 1969). Dispersion of animals may be worthwhile during an epizootic if the disease is focal in nature and associated with an identifiable geographic area. Dispersion of animals away from the site of a disease outbreak is particularly well suited to non-infectious diseases, such as localized toxicity problems, where the animals may be moved from the problem area with no risk of establishing new foci of the disease in previously unaffected areas. When dealing with infectious diseases there is the risk of exporting the agent with the animals and, hence, expanding the spatial distribution of the disease. Assessment of this risk must be based on a sound knowledge of the geographic distribution of the organism or disease and of its ecology. If a disease is known to have a restricted geographic distribution, and particularly when a new disease is discovered in a local area, the method of choice should be containment, and perhaps even total depopulation of the outbreak area, rather than dispersal of potentially infected animals to new areas. However, if the agent is known to be widespread and occurrence of clinical disease is the result of some particular feature of an area, other than simple presence of the causative organism, dispersal may be a sound technique. For example, *Clostridium botulinum* type C, the causative organism of avian botulism, is widespread in the soil of marshes but outbreaks occur only in some marshes and appear to be triggered by local environmental conditions. Hence, there is relatively little risk in dispersing bird from the site of a botulism outbreak.

Dispersion was used during an epizootic of duck plague among wild waterfowl at Lake Andes National Refuge, South Dakota (Pearson 1973). Mortality in the epizootic reached 1,000 birds/day in a population of about 100,000 mallards and 9,000 Canada geese concentrated on a small area of water kept open by an artesian well. Total mortality was approximately 42% among mallards and 3% among Canada geese (Friend and Pearson 1973). The potential management options were to do nothing, to disperse the surviving birds from the area, or to attempt to contain the disease by depopulating potentially infected birds.

In discussing this example, I realize that it is much easier to plan strategy in retrospect than to act in the heat and pressure of an explosive and highly publicized disease outbreak. The factors for consideration included that: (i) at the time, duck plague was considered to be an exotic disease to North America, (ii) this was the first major outbreak recognized in wild waterfowl in North America, (iii) the agent was of high pathogenicity with a high case fatality-rate, (iv) the causative agent was known to be a herpesvirus, which as a group are noted for persistent latent infections, suggesting that survivors would be carriers, (v) the disease was confined to a small geographic area, and (vi) the birds were confined to the site by winter weather but would disperse widely with the onset of spring migration. Arguments against containment and depopulation of surviving birds include the cost both in economic terms and to the continental waterfowl population, the feasibility of depopulation without causing unintentional dispersal, the overall environmental impact of

the methods available for destroying the population, and public reaction to intentional destruction of thousands of birds.

It is difficult to argue for an alternative action when the method used was apparently successful. The outbreak at Lake Andes remains the only major epizootic of duck plague to have occurred in free-flying birds, although smaller outbreaks have occurred and localized occurrences in captive and semi-captive birds have become common (the subsequent occurrences have been caused by strains of virus different from that at Lake Andes). However, I believe that given conditions at the time, containment and depopulation would have been more appropriate than dispersal, and that the need for such drastic action could have been explained to the public in terms of the risk to continental waterfowl populations. Depopulation might have been done by aerial application of a wetting agent as was used to kill waterfowl in an avian cholera epizootic (Pursglove et al. 1976). Movement of the disease as a result of the dispersal operation was impossible to measure quantitatively. Pearson (1973) reported cases of duck plague up to 80 km from the Lake Andes outbreak but it was not possible to tell whether this was the result of natural or induced dispersion.

Dispersion of animals from the site of a disease outbreak may create other problems, e.g., waterfowl could not be herded from the site of some outbreaks of botulism in California because this would have increased depredation on rice crops in the new areas (Rosen and Bischoff 1953). The need for acceptable alternate habitat must be of concern in any dispersal.

Restriction of access by susceptible individuals to a danger area or risk factor, enforced segregation of infected individuals from the healthy portion of the population (quarantine), and reduction of contact between infected and non-infected individuals are all well established techniques for prevention of disease in domestic animals and humans. These have been used to a limited degree in wild populations. Zalm (1986) described a "*waterfowl protection program*" instituted to prevent selenium poisoning of birds at Kesterson Reservoir, California. The program was very similar to that used to disperse birds during an outbreak and consisted of making the area unattractive to waterfowl, hazing birds from the area, and improvement of adjacent habitat to attract birds. The efficacy of the method was not reported, but the hazing program was described to be "*not entirely effective*". Methods of this type were used as to manage anthrax epizootics in Kruger National Park, South Africa (Pienaar 1967). Vultures were thought to be important in disseminating *Bacillus anthracis* from carcasses to watering areas used by game animals. Vultures were dispersed from watering areas, potential roosting sites in trees were destroyed by fire, and excreta of vultures in roosting areas and around watering sites was destroyed by incineration with flamethrowers.

Restriction of access to a site was effective in dealing with an outbreak of bovine tuberculosis in a troop of chacma baboons (Keet et al. 2000). The initial infection of one or more baboons was thought to have occurred through scavenging infected material from African buffalo carcasses. Subsequent

spread within the troop occurred in the tight confines of a small abandoned shelter used by the troop for sleeping. About 50% of the troop was infected, but the outbreak dissipated when the troop was denied access to the building and resumed sleeping in trees. It was concluded that free-ranging baboons are a spillover host for *Mycobacterium bovis* and that the disease could not be maintained without the enhanced level of exposure that resulted from dense congregation in the building.

Factors that affect the success of dispersal operations, such as the difficulty in moving animals and preventing their return, apply equally to deterring animals from using areas. Many techniques have been described for deterring wild animals from using areas to reduce depredation or control nuisance factors, but very little has been published on the use of these techniques for disease prevention. A paper by Boag and Lewin (1980) is notable in that the authors described techniques and measured the effectiveness of various methods for deterring aquatic birds from a risk factor (oil polluted ponds).

12.2.2 Restriction of movement

Enforced separation of infected and uninfected individuals is difficult in free-ranging animals because of their mobility but has been attempted, notably in Africa, to prevent transmission of disease between wild and domestic animals. An interesting historic note is that Masai tribesmen of the Serengeti plains associated occurrence of malignant catarrhal fever in their cattle with calving of wildebeest and traditionally isolated their cattle from the calving areas at that time of year (Jones 1982). In this instance, disease prevention through enforced separation was used for centuries before the causative herpesvirus and the role of wildebeest as carriers of the disease agent were discovered.

A major enforced segregation was the creation of a rinderpest corridor along the border between Tanzania (Tanganyika) and Zambia (Northern Rhodesia) to prevent southward spread of rinderpest in game animals from an epizootic in Tanzania (Vaughan-Jones 1953). Construction of a wooden fence approximately 2 m tall and 128 km in length began in 1941. This was extended to a length of 268 km in 1942. The fence was supplemented by continuous patrols by armed guards situated at 10-km intervals along the fence. They repaired the fence, and "*game breaking through the fence was spoored up and if possible destroyed*". Guards also "*destroyed any game within a workable distance*" of the Zambian side of the fence. Any game could be killed without license in a 32-km-wide zone north of the fence. The life-span of the fence was short, ("*By the close of 1943, the palisade fence had seen the best of its life*") and it was allowed to collapse, but game depopulation continued until 1952. The success of this Herculean effort is difficult to assess. Vaughan-Jones (1953) concluded that the fence had served "*a good purpose*", but the relative value of the corridor and of the "*creation of belts of immune cattle*", that also occurred at the same time (Plowright 1982), in stopping spread of rinderpest is debatable.

Fences have been used in other places to prevent contact between infected wild animals and domestic livestock, and appear to be an effective method for preventing transmission of certain diseases enzootic in wild species to domestic animals. Game-proof fences around Kruger National Park and other smaller parks in South Africa have allowed cattle production in adjacent areas without depopulation of African buffalo infected with foot-and-mouth disease (Jansen 1969) and theileriosis (Henderson 1982). In areas where the "*integrity of the fence cannot be guaranteed*", fencing has been supplemented by vaccination of cattle (Henderson 1982). Domestic swine can be raised in areas where African swine fever is enzootic among warthogs, if the domestic pigs are raised on concrete and contact between wild and domestic pigs is prevented by double fences at least 1 m in height (Henderson 1982). Game fencing was less successful for control of trypanosomiasis in livestock, even when combined with hunting between the game and stock fences (Molyneux 1982). Part of the failure may have been related to the porosity of fences for vector insects and for smaller species of wild animals (Jones 1982). Molyneux (1982) questioned the efficacy and cost-effectiveness of fencing as a control technique for this disease. Veterinary cordon fences of this type may disrupt animal movements that are particularly important for migrating species, such as many species of African ungulate. Owen and Owen (1985) provide a chilling account of the death of thousands of Kalahari wildebeest denied access to water by a fence erected to limit the spread of foot-and-mouth disease to cattle in Botswana. Electrified fences placed strategically in the vicinity of badger setts to which cattle may have access and perimeter fencing around farms to exclude badgers were partially effective in reducing cattle-badger contact in the management of bovine tuberculosis in Ireland; however, the fences were expensive to install (Gormley and Collins 2000). More than 50% of farmers in England who had suffered a tuberculosis outbreak among their cattle indicated that they would never use fencing of badger latrines or setts as a measure to reduce the risk of tuberculosis to their cattle (Bennett and Cooke 2005).

In addition to being expensive to construct, fences and other artificial barriers require continual maintenance and periodic replacement, and are subject to failure. For instance, foot-and-mouth disease spread to cattle outside Kruger National Park in 2000 when African buffalo were able to leave the park after severe flooding damaged the fence (Brückner et al. 2002). A double fence with an animal-free intermediate zone is required to prevent nose-to-nose contact and transmission of some infectious diseases through the fence. The fence also must be capable of restraining movement of all potential hosts or carriers of the disease. A double-fence system, 1.8 m high, used to enclose a wildlife conservancy in Zimbabwe prevented contact between African buffalo infected with foot-and-mouth disease and cattle, but an outbreak in cattle outside the fence was attributed to impala or kudu that transmitted the disease after leaping the fence. The height of game fences was increased to 2.3 m (Hargreaves et al. 2004). If fencing is to be used to control a vector-transmitted

disease, the distance between the parallel fences must be greater than the maximum travel distance of the vector. This is impractical in the case of most arthropod vectors. Construction of fences may require extensive forest clearing or other habitat disruption that may be unacceptable. Fences located in forest must be flanked on either side by a cleared area at least as wide as the height of the tallest tree, so that falling trees do not destroy the fence.

Fencing is probably best suited for preventing access by wild animals to sites contaminated with toxic chemicals or other localized causes of non-infectious disease. Even in such circumstances, fences should be considered as a method of reducing the risk of disease exposure rather than as a way of eliminating such exposure completely.

12.3 Selective removal of diseased animals from the population

The deliberate culling of diseased individuals is used successfully in management of certain types of infectious disease in domestic animals. The objective is to remove infected animals and, in this way, to reduce the amount of infective material available and the likelihood of spread of the disease to healthy members of the population. The method may allow retaining the healthy members of the population and, in some situations, it might be possible to salvage genetic material from culled animals. This technique is an extension of the process of quarantine often used in human medicine, in which individuals with an infectious disease are isolated during the contagious phase to reduce transmission. **Selective culling is best suited for those diseases in which infected individuals can be identified easily and in which the disease spreads slowly through the population**. If only the infected individuals in the population are to be removed selectively, it is necessary to be able to: (i) inspect the population repeatedly, (ii) identify individual animals, (iii) detect infected individuals, (iv) remove infected individuals, preferably early in the course of the disease, and (v) prevent immigration of infected animals from outside the area. It is important to prevent contact between animals that have tested negative and the untested portion of the population until every test-positive individual has been removed.

The efficacy of this technique is dependent on the sensitivity and specificity of the method available for identifying infected individuals. If the detection method has poor sensitivity, many infected individuals will be missed (false negatives), whereas a test with poor specificity may result in unnecessary removal of many healthy animals that react positively on the test (false positives). The process of testing and removal of infected animals is usually referred to as **test and slaughter** in veterinary medicine. It has been used in the control of diseases such as brucellosis and tuberculosis in cattle in many countries. However, even in domestic livestock there have been many

failures to eliminate disease by this method, because of re-emergence of infected individuals among animals that were test negative. This may result from inability to test sufficient animals from the population, tests that are not sufficiently sensitive to detect all infected individuals, or inability to prevent immigration of infected animals.

A basic requirement for selective culling is the ability to handle individual animals repeatedly for testing. For example, in the program to eradicate bovine tuberculosis in Australia, whole herds of cattle were tested repeatedly with a minimum interval of 60 days between tests (Cousins and Roberts 2001). This is the major reason why selective culling has received relatively little attention in free-ranging animals. Test and slaughter was used in conjunction with calfhood vaccination to eradicate brucellosis from wood bison in Elk Island National Park, Alberta (Tessaro 1988) but this population was confined within fenced park boundaries and could be captured easily for repeated testing. Even under these conditions, none of the original animals survived the testing procedure (all eventually tested positive) but some of their offspring were used to develop a disease-free herd. Selective killing of chamois affected with sarcoptic mange was part of a coordinated control program that also included chemotherapy through medicated salt blocks (Pointner 1971). Only animals with visible skin lesions could be detected, so that animals in the early stages of the disease were not detected or removed (in other words, the test used to detect diseased individuals was of low sensitivity).

Test and slaughter was suggested as an alternative to local depopulation as a method to control bovine tuberculosis among badgers in England. However, it was deemed not to be feasible, because there was no suitable method for detecting infection in living badgers (Edwards and McDonnell 1982; Clifton-Hadley et al. 1995).

Annual roundups of African buffalo, with culling of animals that test positive for tuberculosis, have been used in South Africa (Jolles et al. 2005), but the effectiveness of this technique in reducing the prevalence of disease has not been reported.

The feasibility of using test and slaughter to reduce the prevalence of chronic wasting disease in an urban population of mule deer in Colorado has been tested (Wolfe et al. 2004). Deer were captured by chemical immobilization using a dart gun. A biopsy of tonsillar tissue was taken, and the animals were fitted with a radio transmitter and released. Tonsillar tissue was tested for abnormal prion protein and animals that tested positive were located and removed. Prevalence of disease in the group sampled was 8%. It was projected that 5–10 years of annual testing of ≥50% of the population would be required to reduce the prevalence to <2%. It was considered to be feasible to test this proportion of the population annually at a cost of about $300 plus 5 h of personnel time per deer handled. However, this was a "*somewhat unique situation*" in that the deer were habituated to people and readily captured by dart gun, landowners were cooperative, and the urban deer did not interact extensively with other populations affected with chronic wasting disease.

Test and slaughter, with salvage of test-negative animals, was one alternative considered for elimination of brucellosis and tuberculosis from free-ranging bison in the area of Wood Buffalo National Park, Canada (Connelly et al. 1990). The technique was rejected by an environmental review panel (of which I was a member) because it was considered that the diseases could not be eliminated by this method. Factors that led to the decision included: (i) the difficulty of repeatedly capturing ≈3,200 wild bison in a wilderness area of >44,000 km^2 for individual testing, (ii) the need to hold animals that tested negative, together with their offspring, in captivity until it was certain that all bison had been captured and tested, (iii) knowledge that the tests available for detecting tuberculosis or brucellosis in bison were of only moderate sensitivity, (iv) the likelihood that infected animals not detected during testing would infect others within the salvage group held in captivity, (v) the high probability that any recurrence of disease among the salvaged animals would spread rapidly under captive conditions, and (vi) the high probability, based on prior experience with roundups for vaccination of bison, that there would be an unacceptable level of injury and mortality associated with capture and handling. Because of the nature of the tests, each bison would have to be tested repeatedly over several years. There was a certainty that some infected individuals would test negative and be held in contact with the true negatives. In a similar situation, capture and holding of African buffalo for 72 h for tuberculin testing was thought to have increased transmission between infected and uninfected animals significantly (de Lisle et al. 2002). Any recurrence of disease among the salvaged group would necessitate a complete new round of testing and might lead to eventual depopulation of the salvaged animals.

It was recommended that limited salvage of a small number of animals of the desired wood bison phenotype might be done by holding salvaged animals in isolated small groups of 10–20 animals for the repeated testing that would be necessary. The animals salvaged from the wild would never be released, because of the danger of an inapparent infection, but their offspring might be suitable for release after extensive testing. The rationale for this recommendation was that if either brucellosis or tuberculosis reappeared in a small salvage herd, that herd could be depopulated without jeopardizing the entire salvage program.

Selective culling of diseased animals is of limited value for disease management in free-ranging animals but it may be the method of choice for certain diseases in special situations. It might prove useful where it is necessary to control a disease while conserving as much genetic stock as possible within the host population, such as when dealing with threatened species. As this section was written, the state of Wyoming announced plans to conduct a pilot test and slaughter project to reduce the prevalence of brucellosis among wild elk. The animals are available for repeated capture because they congregate annually on artificial feeding grounds.

If the disease agent is highly overdispersed within the host population, (the ‘20/80 rule’, Woolhouse et al. 1997), and if the group responsible for most of the

transmission can be identified, testing or culling without testing, could be targeted at that group preferentially. For example, during the program to eradicate tuberculosis from cattle in Australia it was known that older cattle were a major source of infection, hence, all cattle ≥8 years old were culled. Animals ≥5 years old were culled where the disease prevalence was high (Cousins and Roberts 2001). Because the prevalence of chronic wasting disease in deer increased with age, and adult males were more likely to be infected than females, Greer et al. (2006) suggested that management should include removing older deer from the population and removing males of all ages to prevent spread to new areas. Brucellosis is transmitted primarily through contact with aborted fetuses, placental material and uterine discharges, leading Rhyan and Drew (2002) to suggest that permanent sterilization of bison cows (a form of culling) could be used to greatly reduce transmission of *Brucella abortus*. Even if the sterilized animals were infected, they would be unlikely to transmit the infection.

A variation of selective culling is selective retention of unaffected members of a population as founders for a new disease-free population. As noted earlier, this was suggested as part of the program to deal with disease in bison in Northern Canada. An attempt was made to salvage disease-free wood bison from a wild herd known to be infected with *Mycobacterium bovis* and *Brucella abortus* (Nishi et al. 2001). Calves captured from the wild when they were a few days old were tested immediately for maternal antibodies to *B. abortus*; hand-reared in pairs in an isolation facility, treated prophylactically with antibiotics, raised to maturity in an isolation facility, and used to establish a breeding herd. Calves were captured in 1996, 1997, and 1998. The entire herd was tested repeatedly and intensively and all suspicious reactors were removed. No evidence of either disease was found until 2005, when an apparently healthy 2.5-year-old male born in the facility was killed as part of the herd-management plan and found to have tuberculosis. The likely source of infection was one of the founding calves that, despite having tested negative repeatedly, must have been infected with *M. bovis* at the time of capture (Lutze-Wallace et al. 2006). This example illustrates the difficulty in selecting animals from an infected population when the tests available have only moderate sensitivity. A similar program has been established in Kruger National Park, South Africa to develop a herd of African buffalo free of several diseases (de Lisle et al. 2002).

12.4 Reduction of population density

Reducing the number of animals inhabiting an area has been attempted frequently to manage disease in wild animals. The method is based on an assumption that infectious disease behaves in a density-dependent manner, i.e., that the prevalence of disease increases in populations of increasingly

higher density. If this view is correct, it creates a dilemma for the wildlife biologist for, as stated by Leopold (1939): "*A high density of population—the very thing the game manager is so far seeking—must be set down as the fundamental condition favorable to disease*". [It should be noted that empirical evidence for density-dependence in disease is rare or "*appears to be lacking for wildlife populations*" (Begon et al. 2003)].

The primary goal in reducing the number of animals is to reduce disease transmission. For a disease transmitted directly among individual animals, the rate at which new infections occur is a product of: (i) the contact rate (the number of potentially infectious contacts made by infected hosts per unit time), (ii) the proportion of contacts that are with susceptible hosts, and (iii) the proportion of contacts that actually result in infection (McCallum et al. 2001). It is usually assumed that the contact rate is proportional to animal density, but the actual nature of the relationship between host abundance and transmission is still very much in question. A linear relationship between contact rate and density requires that infected and susceptible hosts mix completely with each other and move randomly within the area (analogous to the mixing of two chemical to which the law of mass action applies). There is considerable debate about whether the mass-action model is appropriate for models of disease transmission and about whether it is animal density or animal numbers that is important. Schauber and Woolf (2003) describe five different "*plausible relationships between host population density and the number of effective contacts per unit time*" that range from constant (i.e., not affected by density) through asymptotic and sigmoid to linear. The actual relationship between the rate of contact/transmission and animal density is unknown for most diseases that occur in wild animals. Some forms of disease transmission, such as disease transmitted by sexual contact, by vectors, or via free-living infective stages may not follow a linear relationship between density and contact rate (McCallum et al. 2001). Obviously, the nature of the relationship between population density and contact rate is critical if management is to be based upon reducing the number of susceptible animals. If the relationship is independent of population size or density, reducing population size or density may have no effect on disease transmission.

Another assumption, which is important when discussing disease management by manipulating animal numbers or density, is that there is a threshold population density or size "*below which infection cannot persist*" (Swinton et al. 2002). An often quoted example of a threshold population ('critical community size') is that a population of 250,000 to 300,000 is required for persistence of measles in human populations (Black 1996). Little is known about threshold populations for persistence or establishment of diseases in wild animals and thresholds may not be abrupt and may be difficult to define in natural populations (Lloyd-Smith et al. 2005). Based on data collected over a 40-year period, abundance thresholds for invasion and persistence of plague in the great gerbil, the main reservoir host in Kazakhstan, have been identified (Davis et al. 2004). Begon et al. (2003) found that the threshold for

cowpox virus in small rodents was determined by the numerical size of the population rather than by population density. Although threshold population size often has been considered to be fixed in a particular situation, Caley and Ramsey (2001) argue that it might be modified if factors that alter contact, such as host behavior, can be manipulated. If transmission occurs within social groups and the size of these groups does not change, reducing the number of groups inhabiting an area changes the overall density but may not change the contact rate within a group (Schauber and Woolf 2003). This may be one factor that allows some diseases to persist in small populations of highly gregarious species. For instance, Dobson and Meagher (1996) estimated that herds of at least 200 bison are required to allow *Brucella abortus* infection to persist, but Joly and Messier (2004) concluded that the minimum number of bison required for brucellosis to persist is likely significantly lower than that number.

Population density might be reduced by removing animals from the area (increasing mortality), by reducing the fecundity of the population (decreasing natality), or by increasing the amount of area available for the animals. The latter alternative is seldom possible, but one suggested action for managing brucellosis in elk in Idaho is development of winter habitat to reduce dependence on artificial feeding grounds that are associated with a high prevalence of the disease (Etter and Drew 2006). Pressure on land for other uses usually precludes increasing the amount of habitat for wildlife. However, this may be the only acceptable method of reducing population density for disease management in certain species. Consider avian cholera in wild waterfowl. Major epizootics of this disease became increasingly common beginning in the 1970s (Brand 1984) at a time when the population of many waterfowl species was at an all-time low. Epizootics usually occur on densely populated wintering or staging areas, and transmission of the agent is probably enhanced by high population density in these foci. The situation has many parallels with the increase in the frequency and magnitude of epidemics in human populations that occurred in the 18th and 19th centuries in association with changes in social patterns and growth of large centers of population as a result of industrialization (Anderson and May 1982). The waterfowl manager is confronted with the dilemma of low overall populations of some species, such as the northern pintail, but high local population density for prolonged periods on the remaining small amount of habitat available to the birds. In this situation, further reduction of the population to control the disease would be unacceptable, and the only acceptable method to reduce bird density would be to create more habitat on these areas and disperse the birds, or to find another way to reduce transmission of avian cholera, and other diseases, under crowded conditions.

Population reduction has been used in an attempt to manage many diseases and the degree of population reduction achieved and the success of such programs has been highly variable. The objective of these exercises is often twofold: (i) to reduce the population density to a level at which transmission

of the disease agent is impaired, i.e., so that on average each case does not replace itself (R_0<1), and (ii) to reduce the impact of other density-related stressors (R_0 applies to a naïve population in which every individuals is susceptible; in a population in which some animals are immune or resistant because of prior exposure, it is better to think of the effective R = R_{eff}). Because each infectious agent has an R_{eff} unique to the particular situation, the degree of population reduction required is different for every disease circumstance. In most cases, population reduction has been done on an empirical basis, often without knowledge of the actual density or size of the population of the wild species, and usually without knowing the degree of reduction that would be required to achieve the desired effect.

The term depopulation is used here in the general sense as synonymous with reduction of the number of individuals. The degree of depopulation may extend to extirpation of the species in an area. In most instances, reduction and depopulation are euphemisms for killing animals, although, if unoccupied habitat is available, excess animals may be translocated to other areas, or the population might be reduced by fertility control. Translocation was used in British Columbia and other areas to reduce the population density of bighorn sheep as one step in the prevention of pneumonia epizootics (Schwantje 1988).

Any attempt to reduce the population of an animal is a race between removal of animals from the population by all causes, including the depopulation effort and disease, and recruitment to the population through reproduction and immigration from adjacent areas. The more intense the depopulation effort, the lower the reproductive rate of the animal, and the lower the likelihood of immigration, the greater the probability of effective population reduction. A consideration in any depopulation effort is that density-dependent effects on recruitment and mortality may reduce the effectiveness of program, so that the population size is not reduced proportionately to the number of animals removed. This may occur through a decrease in natural mortality (so-called compensatory mortality) or an increase in reproductive output (compensatory natality) in response to the reduced population size (Boyce et al. 1999).

Reduction of the reproductive success of a population is a desirable alternative to killing animals as a method for reducing population density but I am not aware of an instance in which fertility control has been used successfully for disease control. Reducing reproduction and delivering antifertility agents to wild animals, particularly pests, have been the subject of intense research for many years, "*but only modest successes have been achieved*" and "*a practical and acceptable method for controlling reproduction in free-ranging wildlife populations has not yet been attained*" (Baker et al. 2004b). Examples of attempted fertility control are available for a number of wild species. Sterilization of 50 and 80% of female brushtail possums reduced the rate of local recruitment but immigration of yearling possums resulted in a stable population on areas studied in New Zealand, leading Ramsey (2005) to conclude

that to be successful in reducing possum populations, sterilization would need to be applied over large geographic areas. Merrill et al. (2006) found that immigration and emigration had a marked effect on the effectiveness of sterilization in reducing a white-tailed deer population and concluded that sterilization is "*unlikely to be a viable means for reducing populations in general*". As a general guide, short-lived species may be more amenable to population reduction through fecundity control, while long-lived species are more sensitive to changes in adult survival (Hone 1999). Wang and Wolff (2003) used the terms "*fecundity sensitive*" and "*survival sensitive*" to describe the relative sensitivity of rodent species to fecundity or mortality types of management. Fertility control was predicted to be less effective than lethal control for managing tuberculosis in badgers because fertility control at maximum only removes one age cohort per year, and because only susceptible animals are removed, whereas lethal control also removes infectious animals (Swinton et al. 1997). In a study of surgically imposed sterility in Australian rabbits, there was a significant decrease in productivity as the level of sterility increased in the population, but that this was "*overcome by increased survival of kittens and adults on the high-sterility sites, such that the base-level numbers of rabbits were maintained*" (Twigg et al. 2000). It was estimated that 60–80% of females would have to be prevented from breeding to result in a long-term reduction in rabbit abundance. Trap-neuter-return programs for feral cats had minimal population effects, because the proportion of the population that needed to be neutered was far higher than was actually achieved (Foley et al. 2005).

The method by which fecundity is controlled, and the effect on animal behavior, may "*help or hinder disease management in wildlife*" (Caley and Ramsey 2001). When 80% of female brushtail possums were sterilized by tubal ligation (which renders them infertile but still sexually active), the transmission coefficient for *Leptospira* infection, that is spread predominantly by sexual contact, **increased** by about 1.3 times the rate in control areas (Caley and Ramsey 2001). R_0 also increased from about 1.5 to 2 and the estimated threshold density for establishment of the infection decreased from 4.4 to 3.4 possums/ha. A method of fertility control that inhibited sexual contact would likely have had the opposite effect.

Population reduction can be of three general types: (i) focal depopulation about a specific site, (ii) depopulation of an area to create a barrier to disease (iii) general depopulation over a large area. Selected examples of use of the three types of depopulation are listed in Table 12.1.

12.4.1 Focal depopulation about a specific site

Four examples in which population density has been reduced in a small area in an attempt to manage disease will be discussed here.

Table 12.1 Examples of the use of depopulation of wild animals as a method of disease management

Species	Disease	Location
Focal depopulation		
Vampire bat	Rabies	Nicaragua[1]
American coot	Avian cholera	Virginia USA[2]
Ground squirrel	Plague	Colorado, USA[3]
Blackbirds	Histoplasmosis	Tennessee, USA[4]
Striped skunk	Rabies	Alberta, Canada[5]
Snails	Polychaete	California, USA[6]
Raccoon	Rabies	Ontario, Canada[7]
Badger	Tuberculosis	England[8]
Deer mice	Hantavirus	Montana, USA[9]
Depopulation to create a barrier		
African buffalo	Rinderpest	Uganda[10]
Red fox	Rabies	Denmark[11]
Vampire bat	Rabies	Argentina[12]
Striped skunk	Rabies	Alberta, Canada[13]
Depopulation over a broad area		
Black-tailed deer	Foot-and-mouth disease	California, USA[14]
Carnivores	Rabies	Alberta, Canada[15]
Mongoose	Rabies	Puerto Rico[16]
Red fox	Rabies	Germany[17]
Ungulates	Rinderpest	Africa[18]
Brushtail possum	Tuberculosis	New Zealand[19]
White-tailed deer	Tuberculosis	Michigan, USA[20]
White-tailed deer	Chronic wasting disease	Wisconsin, USA[21]
Badger	Tuberculosis	Ireland[22]

[1]Gonzalez and Mitchell (1976), [2]Purseglove et al. (1976), [3]Waltermire (1982), [4]White et al. (1985), [5]Rosatte et al. (1986b), [6]Myers et al. (2000), [7]Rosatte et al. (2001), [8]Donnelly et al. (2003), [9]Douglass et al. (2003), [10]Anonymous (1953), [11]Muller (1971), [12]Fornes et al. (1974), [13]Gunson et al. (1978), [14]Anonymous (1921), [15]Ballantyne and O'Donoghue (1954), [16]Toro (1966), [17]Irmer et al. (1981), [18]Molyneux (1982), [19]Caley et al. (1999), [20]O'Brien et al. (2002), [21]Anonymous (2005), [22]Griffin et al. (2005)

Hantavirus pulmonary syndrome in humans usually results from exposure to Sin Nombre virus in and around buildings. Douglass et al. (2003) measured the effectiveness of removing deer mice from ranch buildings as a way of reducing human exposure to infected mice. When resident mice were removed from a building there was: (i) rapid replacement of resident mice by immigrants (often within 2 weeks), (ii) constant turnover in

the population of mice in the building, so that more mice were captured over time than if the residents had not been removed, and (iii) increased probability that infected mice will enter the building. The conclusion was that removing mice from buildings, without making the buildings rodent-proof to prevent repopulation, did not decrease the risk of human exposure to Sin Nombre virus.

Removal of groups of badgers in local areas where cattle were infected with bovine tuberculosis has been used for many years as a management technique in England and Ireland to reduce exposure of cattle to *Mycobacterium bovis*. A large-scale trial began in 1998 to compare the effectiveness of three levels of badger culling (local 'reactive' culling in areas identified on the basis of cattle outbreaks, area-wide proactive culling, and no culling) in reducing the incidence of tuberculosis in cattle herds. Reactive (local) culling was stopped in 2003, because it was associated with a 27% increase in the incidence of tuberculosis in cattle herds compared to the no culling areas (Donnelly et al. 2003). The conclusion was that reactive culling of badgers "*is unlikely to contribute to the control of cattle TB*" and it was thought that culling disrupted badger social organization leading to long-distance movements and dispersal, and increased transmission of tuberculosis among badgers.

Local culling has been used as part of the strategy to control expansion of raccoon rabies from the USA into Ontario, Canada. A three part program of "*point infection control*" was used when single cases of rabies were detected (Rosatte et al. 2001). In this program, all raccoons and striped skunks trapped within a 5-km radius of where a rabid raccoon had been detected were killed. Raccoons and skunks trapped within a 5 to 15-km radius of the site were vaccinated by injection of inactivated rabies vaccine, marked and released. Oral baits containing rabies vaccine were distributed by aircraft over a broad general area surrounding the site. In response to the first three cases of raccoon rabies, 1,202 raccoons and 337 skunks were culled. It was estimated that 83–91% of raccoons present within the 5-km radial areas were removed [this is similar to a plan for control of rabies, if it were to recognized in Britain, which was to cull foxes within a 19-km radius using poison baits (White et al. 1995)].

Corner et al. (2003) followed events that occurred after brushtail possums were virtually eradicated on a 36-ha site in New Zealand. Bovine tuberculosis had been enzootic in possums on the site for several years prior to the population reduction. The population of possums recovered rapidly, reaching a peak of 167 animals 30 months after depopulation. During the 40-month study period, 370 possums were detected on the area. The first case of tuberculosis was detected 4 months after depopulation; in total, 30 cases of tuberculosis were detected in the study period. Four different types of *M. bovis*, based on restriction endonuclease analysis, were found. It was concluded that re-emergence of tuberculosis resulted from reintroduction of the agent by infected adult animals from adjacent areas

expanding their range into the depopulated zone, and by immigration of dispersing infected juveniles.

12.4.2 Depopulation of an area to create a barrier to disease

Efforts to control rabies in wild animals provide some of the best examples available of the use of depopulation as a technique for management of disease. The control of vampire bat-transmitted rabies illustrates the evolution of technique from attempts at general population reduction (unsuccessful) to precisely targeted, intense control to create a barrier to the disease. Rabies transmitted by vampire bats is a problem throughout the range of these bats from Mexico to Argentina and on some Caribbean islands. Fornes et al. (1974) estimated that 500,000 cattle died annually of rabies in the region. European settlement favored vampires through provision of an abundant food base of large animals, where there had been few or none, and of roosting sites in mines, tunnels and wells. Lord (1980) concluded that there were "*probably many more*" vampires in the region at the time of writing than prior to settlement. The major method of control of the disease in cattle was through vaccination but this had no effect on the disease in bats, and rabies continued to be a risk to unvaccinated humans and animals.

Early attempts to control vampire bats were largely ineffective, e.g., destruction of bats at roosts had been done in Trinidad for many years "*with not the slightest indication of a fall in the population*" (Williams 1960), despite the fact that adult female bats produce only one young each year. Development of effective control methods depended upon research in two general areas. The first was using an understanding of bat biology in development of sophisticated ways for killing bats. Early methods took advantage of the habit of vampires of reopening partially healed wounds on cattle. Wounds were painted with "*strychnine syrup*" (Williams 1960) or with anticoagulants, resulting in the death of bats that returned to feed. This was refined using the discovery that anticoagulants could be administered safely to cattle, either intra-ruminally or parenterally, resulting in the death of bats that fed on blood containing the systemic anticoagulant (Lord 1980). Linhart et al. (1972) described a method that amplified the impact of poisoning on the bat population. Bats captured alive were painted with anticoagulant in petroleum jelly and released to return to the colony, where 20–40 other bats would die from toxin ingested during grooming of the painted individual (Lord 1980).

The second breakthrough was in recognition, through surveillance and examination of records of bovine cases, that rabies occurred as migratory epizootics at about 4-year intervals. Waves of disease moved across the landscape in a predictable direction and at a relatively slow rate of about 40 km/year (Lord 1980). Ahead of the epizootic the virus was absent from bats and few or no bats had immunity. At the epizootic front many bats became infected; some died and some became immune. Behind the wave many bats

were immune and the disease apparently disappeared because of the low density of susceptible animals. This information was used to control an epizootic among cattle in Argentina through local depopulation of vampires in advance of the disease (Fornes et al. 1974). An area 50 × 30 km was selected in front of the epizootic, in which all bats were roosting in water wells. About 95% of roosts in the area were identified and the population of vampires in the area was measured prior to treatment. All known roosts were gassed with cyanide and then closed with bat-proof screening. Based on recapture of bats marked and released prior to gassing, the population was reduced by about 95%. By comparing the progress of the rabies epizootic in the treated area to that in adjacent untreated areas, the method was judged to have been effective in blocking spread of the disease.

Lord (1980) described further refinements and stressed that the control effort must be located 10–20 km in advance of the latest bovine case, because the outbreak in vampires had already passed and was in "*recess*" where cattle were dying. Lord recommended that the control area should extend 15 km to each side beyond the width of the advancing front of disease in cattle, and to a depth of 15 km in advance of the front, based on information that the home range of the bats was less than 15 km in diameter. Within the treatment area, groups of cattle were held in corrals, located at 5-km intervals, for at least a week to attract and "*entrain*" local vampires. Bats captured with mist-nets at these sites were painted with anticoagulant and released to poison other bats in the roosts. Although the degree of population reduction attained by this method was not measured, the technique was reported to have eliminated rabies outbreaks in Brazil and Venezuela.

There are many parallels between attempts to control rabies transmitted by vampire bats and efforts to control the disease in areas where terrestrial wild species are involved. Rabies in Europe is transmitted primarily by the red fox and the disease spread across Europe as an epizootic wave that moved at a rate of 27 km/year (Bogel et al. 1976) or 30–60 km/year (Toma and Andral 1977). Passage of the wave was followed by periods of quiescence interspersed among smaller waves that occurred at 2 to 5-year intervals. MacDonald and Voigt (1985) concluded that fox depopulation had little effect on the speed at which the epizootic spread. Hunting was ineffective in reducing the fox density sufficiently to impede the disease (Wandeler et al. 1974). Muller (1971) estimated that shooting for bounty only reduced the population by about 25%. Intensive gassing of dens resulted in a much more dramatic reduction in population, particularly when accompanied by mortality as a result of rabies but, even after intense gassing operations, the epizootic rebounded in some areas (Wandeler et al. 1974). Very intense local depopulation of foxes to create a barrier, using gassing of dens as a major technique, was apparently successful in preventing an epizootic from entering a disease-free area in Switzerland (Wandeler et al. 1974) and in restricting, and eventually eliminating, the disease after it entered Denmark in 1964 and 1968 (Muller 1971). In these situations the disease was entering an unaffected area from one

direction as a wave and lateral or flanking movements by the disease were precluded by geographic barriers (mountains in Switzerland, and by the narrowness of the isthmus linking Denmark to the enzootic focus of rabies in Germany). Gassing of dens and shooting for bounty in the control zone in Denmark was estimated to have reduced the fox population by 80%, to a density of 0.2 foxes/100 ha. Muller (1971) suggested that the control zone in front of an advancing wave of rabies in foxes should extend to a depth of 60–100 km. When rabies re-entered Denmark in the same general area in 1977, gassing of dens and bounty shooting were supplemented by rewards for reporting occupied dens and by use of strychnine poisoning within a 60-km-wide zone (Westergaard 1982). The width of the zone had to be extended beyond 60 km in one area.

Depopulation has been used to arrest an epizootic of rabies, primarily in striped skunks, that spread across the Canadian Prairie Provinces. This epizootic began as an extension from the adjacent northern USA about 1959 and spread progressively northwestward across Saskatchewan between 1963 and 1970, reaching the border with Alberta about 1970 (Gurba 1974). In 1971, following diagnosis of rabies in two skunks in Alberta, skunk depopulation was begun within a 30-km-wide zone adjacent to the border with Saskatchewan and stretching approximately 635 km north from the US and Canadian boundary. Depopulation was by poisoning, shooting, and trapping, but the extent to which the population was reduced is unknown. Depopulation of skunks also was done within a 5-km radius of the location of any rabid skunk found beyond the control zone. A similar program was instituted along the border with Montana when rabies became established across that state (Pybus 1988). The program was judged to have been successful in limiting the establishment and spread of rabies in skunks in Alberta compared to neighboring jurisdictions (Gunson et al. 1978; Pybus 1988). When rabies has occurred beyond the depopulation zone, intense local depopulation has been used (Rosatte et al. 1986b; Pybus 1988; Hutchings 1992).

Use of local or barrier depopulation for disease control depends on effective surveillance and rapid reporting of cases of disease, so that the control measure can be applied promptly in the correct location. If recognition or reporting are delayed, the disease may move through an area or spread beyond a radius at which the population can be controlled effectively. Surveillance of diseases in wildlife at a level useful for barrier control is extremely difficult. Most cases of disease go unreported, e.g., it has been estimated that only 2–10% of foxes dying of rabies in Europe were reported. Bacon (1981) addressed the consequences of delayed recognition and a low reporting rate on the probable effectiveness of programs to control rabies, should it become established in foxes in Great Britain. He concluded that if the probability of a single diseased animal being reported was about 5%, the first reported case could actually represent a "*fairly late stage in a rapidly developing epizootic*". Bacon predicted that if the reporting rate was 2%, and if there was a 95% probability that the disease was eventually reported,

4–6 months might elapse before the first case was reported, during which an estimated 148 foxes might have become rabid and the disease might have spread over a radius of 30 km. Effective population control on such a large area (>2,800 km^2) would be difficult. Hone and Pech (1990) modeled the possible introduction of foot-and-mouth disease into feral pigs in Australia. They concluded that the probability of detecting an individual case of the disease with the existing surveillance system was less than 0.0015. They estimated that between 28 and 3,077 cases might occur among feral pigs before the disease was detected.

12.4.3 General depopulation over a large area

Local depopulation, such as about a single case of a disease recently introduced to an area, or barrier depopulation ahead of an advancing wave of disease usually are short-term programs with a limited objective of stopping or stamping out the disease quickly. Large amounts of effort can be concentrated in a small area. In contrast, depopulation over large areas requires a long-term commitment in which the population must first be reduced, and then population growth must be stopped. Holding the population abundance at the reduced level may require stopping the higher population growth rate, due to compensatory mortality or natality, which may occur in the reduced population. If the disease is a significant mortality factor in the population, a reduction in disease prevalence also may have a significant effect on the population growth rate, e.g., tuberculosis at 2% prevalence was estimated to reduce the steady-state population density of brushtail possums to 74% of the carrying capacity in the absence of the disease (Roberts 1996). If the goal is to eradicate the disease, it is necessary to know the minimum or threshold population density at which the disease can persist (assuming that transmission occurs in a density-dependent manner) and the rate of culling that is necessary to keep the population below that density.

It is difficult to assess the efficacy of many population-reduction programs done in the past because few projects attempted to evaluate the effect of depopulation. A danger in evaluating the effect of depopulation done during a disease outbreak is that of overestimating the impact of the control program, by ascribing the reduction or disappearance of disease to depopulation, when it may have been the result of the normal extinguishment expected in the latter stages of an epizootic (this has been termed 'sliding to glory on the downward slope of a declining epizootic curve'). An epizootic of rabies that swept into the western provinces of Canada from the Northwest Territories in 1952 provides an example of the difficulty in assessing the effect of depopulation. Massive depopulation of wild carnivores was conducted in Alberta between 1952 and 1954 using poisons (including 30,204 cyanide guns), trapping, shooting, and hunting from aircraft. The estimated kill in the forested area of the province was 50,000 foxes, 35,000 coyotes, 4,200 wolves, 7,500 lynx and

1,850 bears; 60,000–80,000 coyotes was considered a conservative estimate of the kill in the agricultural area of the province (Ballantyne and O'Donahue 1954). The "*apparent eradication*" of rabies in southern Alberta was credited to "*the drastic depopulation of predatory animals*", together with vaccination of owned dogs and killing of stray dogs (Ballantyne and O'Donaghue 1954). However, "*extension of the same epizootic into Saskatchewan, Manitoba and British Columbia, diminished despite the lack of special program of wildlife control*" (Tabel et al. 1974) and the disease disappeared in these neighboring provinces. The epizootic also spread into northwestern Ontario where it "*petered out*" spontaneously without any depopulation of potential hosts (Tabel et al. 1974). Thus, the epizootic followed a similar course in adjacent jurisdictions, one of which undertook population control and four of which did not.

In many large-scale depopulation programs, all that is available is a subjective appraisal of the efficacy and speculation about what might have been without the program. One notable success may have been the eradication of foot-and-mouth disease from deer in California. This required a very intensive effort and the slaughter of 20,698 deer over about a 1-year period (Anonymous 1921). Other programs, such as encouraging trapping to reduce rabies in foxes, seem to have had equivocal success at best (Linhart 1960; Marx 1966; Davis 1974) and extensive attempts to control rabies in Europe through reduction of the fox populations appear to have been ineffective (Irmer et al. 1981; Anderson et al. 1981).

Depopulation of wild species over large areas has been done in an attempt to control bovine tuberculosis in cattle in Ireland, New Zealand, and Michigan, USA. The three situations are very different because the badger is a protected species in Ireland, the brushtail possum is a threat to conservation values (Warburton and Thomson 2002) as well as to domestic animal health in New Zealand, while the white-tailed deer is a valuable game animal in Michigan.

There have been two trials to measure the effect of depopulation of badgers on the incidence of tuberculosis in cattle herds in Ireland. In the East Offally project, the results of badger depopulation on a 738-km^2 area were compared with those from a 1,455-km^2 surrounding control area. The risk of tuberculosis occurring in cattle herds was significantly greater in the control area than in the depopulation zone (O'Mairtin et al. 1998), despite immigration of badgers from the control zone into the depopulation area (Eves 1999). The second study, done from 1997 to 2002, involved sites in four geographical regions and comprised 3.9% of the agricultural land of the Republic of Ireland (Griffin et al. 2005). Each study site contained a 'removal' area and a 'reference' area in which badgers were removed locally in response to severe outbreaks of tuberculosis in cattle. The average number of badgers killed during the first 2 years was about eight times greater on the removal than on the reference areas (0.57/km^2/year versus 0.07/km^2/year). In the final year of the study there was a 60–96% decrease in the rate at which tuberculosis was

confirmed in cattle herds in the removal areas compared to the reference areas. The conclusion was that "*Although feasible, we acknowledge that widespread badger removal is not a viable strategy for the long-term control of tuberculosis in the Irish cattle population*" (Griffin et al. 2005).

Bovine tuberculosis occurs in possums over about 38% of New Zealand (10 million ha), of which about 4.6 million ha were under sustained possum control in 2002 (Anonymous 2002). Various models have predicted that without immigration, tuberculosis would not persist in populations of possums held below about 40% of the habitat's carrying capacity (Barlow 1991a, 1991b, 1993), that tuberculosis could be eradicated from a population of possums by reducing the density to 43% of the no-disease carrying capacity (Roberts 1996), and that tuberculosis will die-out in possum populations within 5–10 years if the population can be reduced by about 70% and held at that level (Ramsey 2000). In an early trial, the incidence of tuberculosis in cattle declined after >70% of the possums in the area were killed and the population was then maintained at that level (Tweddle and Livingstone 1994). Caley et al. (1999) examined the effect of reduction of possum numbers on the prevalence of tuberculosis in possums and the incidence of tuberculosis in cattle on 12 farms. Where possum abundance was kept to <40% of pre-control abundance over a 10-year period there was "*a major reduction in both the cumulative yearly incidence of Tb in cattle, and the prevalence of the disease in possums*", although immigration of possums from surrounding areas was not prevented. Whether a level of culling of possums sufficient to achieve control or eradication of tuberculosis can be attained over the huge area affected by tuberculosis in New Zealand remains an open question.

Bovine tuberculosis has been recognized in a 650-km^2 focus in Michigan since 1994 (Miller et al. 2003). Deer density in the area reached 19–23/km^2 as a result of low hunting pressure on females and extensive supplemental feeding (O'Brien et al. 2002). Beginning in 1998, restrictions were placed on baiting and feeding deer, hunting seasons were lengthened, and unlimited permits were available to kill antlerless deer in an attempt to reduce contact and deer density. The deer population was halved (de Lisle et al. 2002) and the apparent prevalence of tuberculosis in deer declined by about 50%; however, the decline in apparent prevalence "*cannot yet be definitively attributed*" to the control strategies (O'Brien et al. 2002). It was "*perhaps ominous*" that after an initial decline and despite concerted effort, the prevalence of tuberculosis in deer remained flat and did not continue to decline (O'Brien et al. 2002). Schmitt et al. (2004) observed that aggressive reduction of the deer population is unacceptable to many hunters and landowners, and that it may not be possible to sustain, let alone increase, hunting pressure to further decrease deer density. A field trial was conducted in 2003 to assess the potential for selective culling of infected animals as a "*less efficient (i.e., more costly) but potentially more socially acceptable alternative*" to general depopulation (Schmitt et al. 2004).

During the past decade, chronic wasting disease (CWD) has been found in wild cervids in a continually enlarging area of North America. It is assumed that CWD behaves similarly to other chronic transmissible diseases and that high animal density is associated with increased transmission and prevalence. Several jurisdictions are attempting to reduce deer density in areas with a high prevalence of CWD. The most aggressive depopulation has been in Wisconsin (Anonymous 2005). Two "*disease eradication zones*" of about 3,350 and 1,370 km^2 were established in 2002, in which the goal is to reduce deer density to <2 deer/km^2 through increased hunting (extended seasons and liberal bag limits, out of season permits to landowners, cash awards to hunters that kill a CWD affected deer) and shooting by government sharpshooters. The average density of deer was reduced by about 35% in the core area between 2003 and 2005, and density was reduced from 18 to 11 deer/km^2 (40% reduction) in an area with the highest population density prior to culling. The eradication zone is surrounded by a 64-km-wide herd reduction zone in which the goal is to reduce the density to <4 deer/km^2. The number of deer killed in this zone increased from 41,500 in 2002–2003 to 54,800 in 2004–2005. When this was written it was not known if the number of deer can be reduced to the target density or what density is required for the disease to persist. Because of difficulty in measuring change in the prevalence of a disease that occurs at low prevalence, it may be "*an additional 4-6 years before enough information is available that we can reliably determine if the CWD control program is effectively reducing prevalence and size of the affected area*" (Anonymous 2005).

12.4.4 Extirpation

The ultimate extension of depopulation is extirpation of one or more species from an area. Although organized game destruction was used in Africa for control of trypanosomiasis (Molyneux 1982), it is unlikely that total elimination was accomplished, except where major habitat destruction also occurred. Eradication of free-living animals has been proposed in the event of introduction of a serious foreign animal disease, such as foot-and-mouth disease to a disease-free area (e.g., Hone and Bryant 1981; Pech and Hone 1988) but the ability to accomplish extirpation of a free-living animal seldom has been tested. Eradication of foxes that were introduced, together with *Echinococcus multilocularis*, to Rebun Island, Japan may be an example of successful management of a disease through eradication of a wild species (Ito et al. 2003). Bomford and O'Brien (1995) proposed six criteria for deciding if eradication is feasible and preferable to ongoing removal of a portion of the population. The first three of these are essential for achieving eradication; the remainder are desirable and determine whether eradication is the favourable option: (i) the rate of removal must exceed the rate of increase at all population densities, (ii) immigration must be prevented, (iii) all reproductive

animals must be at risk, (iv) animals can be detected at low densities, (v) benefit-cost analysis favors eradication over continued control, and (vi) there must be a suitable socio-political environment.

The first criterion seems obvious, but there are two complicating factors. The first is that when a population is reduced there may be compensatory changes in mortality or natality so that the rate of removal (as a proportion of the population) may have to increase. The second factor is that as a population is reduced, the effort required for further reduction escalates exponentially. Elimination of the last few animals may be extremely difficult. Choquenot et al. (1998) examined data from programs that used shooting from helicopters for control of feral pigs in Australia. At high pig density, about 60 pigs could be killed per hour, but the hours/kill increased exponentially as the pig population was reduced to approximately 2–6 animals/km^2. Saunders and Bryant (1988) found that some pigs learned to avoid helicopters and concluded that "*eradication of feral pigs during an outbreak of exotic disease may be an unrealistic goal. . . .*" Eliminating the last 1 to 10% of any population may require as many resources as eliminating the first 90–99% (Myers et al. 1998). The cost per animal removed may lead to a loss of commitment to completing eradication, particularly if the disease seems to have disappeared.

Of the few attempted eradications of vertebrates that have been successful, most have involved introduced species on islands, e.g., the foxes on Rebun Island, or other situations where reintroduction is very unlikely. Many other attempts have failed because of addition of animals through immigration. For example, Forsyth et al. (2003) predicted that feral goats could be eliminated in the short-term from a large national park in New Zealand by different methods but that annual immigration would prevent eradication under all scenarios.

Not every animal in a population needs to be eliminated for eradication to be successful in the long term, but each and every female capable of reproduction must be removed (Myers et al. 1998). One factor considered when exploring the possibility of extirpating free-ranging bison affected by tuberculosis and brucellosis in northern Canada was that females are intensely gregarious and, hence, relatively easy to locate and trap, whereas many older bulls are solitary and difficult to find. However, finding these solitary males need not be a priority if the females can be eliminated.

The ability to detect animals at very low density is important and may require the use of techniques such as the release of Judas animals (Campbell et al. 2005), marked with radio-transmitters, that will search out the remnant animals. Costs for monitoring the population will increase as the density decreases and as complete extirpation is neared it may be very difficult to determine when and, if, elimination has been accomplished. In campaigns to eradicate insect pests: "*pressure often exists to declare successful eradication*" (Myers et al. 1998), so that there is premature conclusion of a nearly successful program. This also is likely to occur in attempted extirpation of a wild vertebrate.

Criteria (v) and (vi) described by Bomford and O'Brien (1995) apply to all disease-management programs. Myers et al. (2000) listed additional requirements for a successful eradication program including sufficient resources committed to the program to see its completion, and clear lines of authority that allow all necessary actions to be taken.

A program to eradicate approximately 100–200 introduced passerine birds resident in San Diego, California illustrates the difficulty in eliminating even a small population of a sedentary species (Van Way 1984). After 3.5 years, during which 330 birds were killed at a cost of $212/bird, the population was estimated to be about a dozen birds and "*whether eradication is feasible*" was still to be determined. However, muskrats were eradicated from Great Britain by trapping (Warwick 1940) and coypu were eradicated from England using live traps (Gosling 1989).

Hone (1983) tested the feasibility of eradication of feral pigs on a 50-km^2 area in Australia, as might be required should foot-and-mouth disease be introduced. After an intense program of poisoning with sodium fluoroacetate (1080) followed by shooting from helicopters, it was concluded that "*eradiation would be almost complete, but probably not entirely so*" on this small area in the short term. Rates of recolonization through influx from adjacent areas and the cost of maintaining the depopulated status of the zone were not examined. Pech and Hone (1988) suggested that elimination of >95% of feral pigs within a period of <21 days would be needed for rapid disease eradication in the event of introduction of foot-and-mouth disease, although a somewhat lower rate of population reduction might be successful in the long term.

Extirpation is more feasible for large, conspicuous species with a low reproductive rate that inhabit open country than for small cryptic species that reproduce rapidly in forest habitat. However, elimination of even very large animals may be difficult. The feasibility of eliminating feral Asian water buffalo on a 389-km^2 area in Australia was tested as a technique that might be used in the event of a foreign animal disease incursion (Ridpath and Waithman 1988). The population was reduced by >97% within 3.5 months by a combination of helicopter roundups, shooting from helicopters, and shooting from the ground. Removal of the remaining animals became so difficult and expensive that it was concluded that: "*eradication of buffalo from their entire range would be an unrealistic objective, both economically and practically*". However, during a national campaign to eradicate tuberculosis, the population of buffalo was reduced sufficiently to lead to disease eradication, i.e., the disease and not the buffalo was eliminated. In 1984 only about 5,000 of the estimated 350,000 buffalo in the Northern Territory of Australia were in fenced enclosures. The population was reduced to 30,000 to 40,000 by round-ups using helicopters (aerial mustering) and shooting from helicopters (Cousins and Roberts 2001). Total depopulation of cattle and buffalo was done in large areas using these techniques, plus the use of Judas animals released to seek out the few residual animals. Judas animals were relocated by radiotelemetry and buffalo that they had joined were destroyed.

Elimination of free-ranging bison in and around Wood Buffalo National Park, Canada, was recommended as the only feasible method of eradicating bovine brucellosis and tuberculosis from the area (Connelly et al. 1990) [recall that brucellosis may persist in a population of ≤200 bison (Dobson and Meager 1996; Joly and Messier 2004), and the difficulty in trying to select tuberculosis-free bison from an infected herd (Lutz-Wallace et al. 2006)]. Elimination was to be followed by restocking with disease-free wood bison. Elimination was judged to be feasible because of the susceptibility of the major portion of the population to trapping during winter (particularly the females), the relatively low reproductive rate of the bison, the presence of wolves that would aid in depopulation, and the high visibility of bison tracks and feeding craters in snow during winter that could be used to locate animals from the air. It was recommended that the area be left free of bison for several years after removal of the last animal, with frequent aerial surveys to ensure that no animals remained, prior to restocking with disease-free bison.

12.4.5 Depopulation in review

Some general features are evident from the examples in Table 12.1 and other published accounts of attempted disease management through depopulation. The first is that this type of management usually has been used to control carnivores and species, such as bats and rodents, considered to be pests. Depopulation seldom has been attempted against game animals or other species considered beneficial to humans. This likely reflects difficulty in convincing the public of the wisdom of removing or killing desirable species.

A second feature is that it is more effective to prevent entry of a disease into an area through local radial or barrier depopulation than it is to try to reduce an established disease. Disease management is analogous to water management in this regard. It is easier to quell an advancing flood or to drain a small pothole while standing on dry land, than it is to drain a lake while having to tread water to stay afloat. In addition, local or barrier depopulation is usually short term, so that intense effort can be concentrated in time and space.

Population reduction, without any change in the habitat is, at best, a temporary measure. To be effective for any length of time there must be continued depopulation at a level sufficient to overcome population growth. If the population is reduced below the carrying capacity of the habitat the population will rebound, unless the carrying capability is also reduced. Recovery will be more rapid if there is immigration from surrounding areas. Following are a few examples of the rate of recovery after population reduction. Waltermire (1982) followed a population of Richardson's ground squirrels in a campground that was reduced by about 90% through shooting in the spring for plague control. By mid-summer the degree of reduction of population was 52–64% compared to control areas and some degree of reduction persisted for 1 year. It was concluded that effective population control for plague

management "*requires at least a 90% reduction*" of the total population. Recruitment through immigration of animals from adjacent areas was very important in this example. Layne and McKeoun (1956) estimated that a red fox population in New York State would have to be reduced by >64–76% to bring about an actual reduction in the population in subsequent years. Populations of European fox reduced to 20–30, 40, 60 and 80% of the original population for rabies control were estimated to recover to original density within 4, 3, 2 and 1 year(s), respectively (Bogel et al. 1974). These estimates were based upon large geographic areas in which immigration would not be a significant recruitment factor. A poisoning campaign for the control of rabies on Grenada destroyed one-third to two-thirds of the mongoose population in some areas, but the population was thought to have recovered within 6–9 months in most areas (Everard and Everard 1985). A population of Australian bush rats recovered to pretreatment levels within 2 years after large numbers of animals were removed experimentally (Lindenmayer et al. 2005), largely through reproduction by residual animals that escaped capture.

Based on current information, I believe that it is unlikely that populations of popular game animals, such as deer, can be reduced sufficiently for management of diseases such as tuberculosis or CWD by voluntary hunting. Factors that reduce the effectiveness of hunting include that (i) severe population reduction is unacceptable to many hunters and landowners, (ii) a limited number of hunters are willing to harvest antlerless animals (including females that have the greatest impact on population size), (iii) hunters lose interest when deer density falls and hunting becomes less successful, (iv) the number of hunters is declining in many jurisdictions, and (v) land owners become intolerant of large numbers of hunters on their property. Many of the same factors probably apply to removal of furbearers by trapping.

My final observation is that methods that may be effective in reducing populations, such as widespread use of poison and aerial shooting, have low public acceptance in many areas of the world.

12.5 Summary

- Dispersion of animals may be used to manage disease if: (i) the disease is localized to a specific site, (ii) there is little risk of spreading the causative agent to new areas, (iii) suitable alternate habitat is available, and (iv) the animals can be induced to move and to stay in the new location.
- Methods to affect disease management through manipulation of either the distribution or density of wild animals are most successful when they can be applied intensively on small geographic areas.
- Fences and other artificial barriers to restrict animal distribution or movement are expensive, require continual monitoring and maintenance, and are subject to failure.

- Selective culling of diseased individuals (test and slaughter) has limited usefulness in wild animals, except in special circumstances, because of difficulty in capturing individuals for repeated testing, lack of sensitive tests, and the need to prevent contact between animals that test negative and untested individuals.
- If one identifiable group within the population is responsible for most disease transmission, they might be targeted for selective management.
- Focal depopulation, as about cases of a recently introduced disease, or as a barrier to a spreading wave of disease may be effective, but requires intense surveillance and rapid identification of cases to be effective.
- General population reduction over large areas likely will require a huge commitment of resources over a long time period to be successful in controlling or eradicating disease. The effort and cost/animal removed will increase exponentially as the density of animals declines. Encouraging increased trapping or hunting over large geographic areas is unlikely to result in population reduction to the level required for disease management.
- Methods to reduce population density of animals through impairment of reproduction have not been used for disease control, but might be appropriate in some situations, particularly for short-lived species with a high reproductive rate.
- Reduction of animal numbers in an area either through dispersal or depopulation requires an intensive effort and the effect is temporary, unless habitat is made less attractive to the species. The speed of repopulation is related to the reproductive potential of the animal and the rate of influx from adjacent areas.
- Total elimination or eradication of an animal species for disease control will be difficult to accomplish, except through a very sustained, intensive program, or in conjunction with modification of habitat suitable for the animal.

13 Disease management through treatment and immunization

> "*There exists a discrepancy between our knowledge of how to treat an individual and how to control the infection within a community*"
>
> (Anderson 1982)

In the developed world, our perception of infectious disease in humans has changed dramatically within the past half-century as a result of advances in chemotherapy and effective mass immunization. Many diseases that occurred regularly and were feared greatly only a few decades ago are now so uncommon that they are viewed almost as medical curiosities. Most of these changes have occurred within my lifetime. For instance, my grandmother died of tetanus at about the time I was born; this disease is now prevented through routine immunization. I distinctly remember school being cancelled and the holiday beginning early one summer because of an outbreak of poliomyelitis. This was the last epidemic of poliomyelitis in the area but I vividly recall attending the funeral of a classmate victim of the disease that summer. Poliomyelitis has virtually disappeared as a clinical entity in many parts of the world because of routine prophylactic immunization. Other diseases are now dealt with routinely by antibiotic treatment or other chemotherapy. For example, I was pleased to find that the case/fatality rate in chlamydiosis (psittacosis) had declined significantly from the pre-antibiotic era when I acquired the disease from wild birds a few years ago.

A similar change has occurred in veterinary medicine. Many significant infectious diseases of domestic animals can now be prevented or treated satisfactorily. When serious disease problems do occur, they usually result from failure to use the methodology available, or because the cost of control or preventive measures is judged to be economically unsound, or because a new disease has emerged. Most infectious diseases that occur in wild animals could be prevented or treated satisfactorily in the **individual animal** using the same techniques used in humans and livestock; however, there are few situations in which these techniques can be applied satisfactorily to an entire free-living **population**. The problem usually relates to delivery of the treatment or vaccine rather than to efficacy of the treatment if it could be delivered.

In this chapter I will review some methods that have been used with varying degrees of success to treat sick wild animals or to prevent illness through

immunization. Control of vectors of disease, e.g., through the use of pesticides to control fleas on rodents during epizootics of plague, and treatments used to block transmission, do not fit within my definition of therapy and have been discussed earlier.

13.1 Therapy of diseased animals

Leopold (1939) stated that: "*doctoring*" [by which he meant treatment] "*is of recessive importance in health control*" for all species. I agree with this general principle (except, of course, when I have a personal interest in the results of the doctoring!); however, there may be instances in which treatment can be an effective part of an overall disease-management strategy. Such instances might include:

- outbreaks in which it is possible to treat and save a large number of animals
- intensively managed or rare species in which attention to the individual animal is possible and justified
- treatment as a part of the preconditioning process prior to translocation
- treatment or rehabilitation for reasons other than any effect of the saved individuals on the population

In many disease conditions of wild animals, sick individuals seldom are detected until the disease is in an advanced stage. For example, in avian cholera epizootics, it is unusual to find even a single sick bird, although hundreds may be dying in the area. In such instances, treatment is of no practical benefit. In other situations the morbidity is so dispersed in time and space that it is impractical to collect the sick animals for treatment. In a few circumstances, such as outbreaks of avian botulism or oil spills, one may be confronted with many affected animals in a small area. The decision to treat or not to treat in such circumstances should be made on some rational basis. Among the factors to be considered are:

- the biological significance of the individuals that might be saved
- the probability of success; i.e., the proportion of the treated individuals that will make a complete recovery
- the cost of the treatment
- the availability of facilities and personnel
- the consequences of not attempting treatment and, conversely, the indirect benefits that may result from a treatment program

The first three factors can be considered together because they involve placing a value on the individuals saved. In most cases, one must assume that funds expended for treatment will be taken from some other program. Thus,

funds expended for treatment may be lost to habitat improvement or some other management program that also would benefit the species. In general, **the number of animals of a common species that can be saved in any disease occurrence will not have a significant effect on the overall population**. However, this may not be the case for local sub-populations or for rare species that could be decimated by a single disease incident. Cairns and Elliot (1987) suggested that in assessing the risk of oil spills to seabirds, consideration should be given to the possibility that entire colonies could be killed and that population recovery in such instances would be very slow. In some such instances, treatment to salvage a part of the local population might be justified.

Even for common species, their population status should be considered in evaluating the feasibility of treatment. For example, the population decline of some species of waterfowl, such as the northern pintail and lesser scaup, might influence the way we evaluate the role of treatment in disease management, notably in outbreaks of avian botulism. At the time of writing the first issue of this book (1992), the number of mallards and pintails in North America was near the lowest level since systematic surveys began in 1955. The annual cost (in terms of dollars/duckling fledged) of various management procedures to increase duck production in the Dakota pothole region varied from \$2 for predator control to \$223 for construction of islands (Lokemoen 1984). The estimated cost of various habitat-management procedures proposed for Saskatchewan ranged from about \$8 to \$23 per additional duckling fledged (Anonymous 1988). If the same cost/benefit analysis were applied, it might be economically sound to treat and save ducks during botulism outbreaks, particularly when most of the affected birds are adults with an otherwise good life expectancy.

The cost per individual saved in any treatment program is influenced by the success rate and cost of the operation. There has been relatively little study of the cost of such operations, but experience with oil spills may be instructive. Early efforts to save oiled birds were largely chaotic and unsuccessful (Hay 1978) with mortality exceeding 80% (Dein and Frink 1986). However, by using techniques refined through experience and research, the mortality rate has been greatly reduced to about 15%. Triage, to select those individuals most likely to benefit from treatment is very important in oil-spill incidents, so that the effort is applied in the most effective manner (Lauer et al. 1982). This principle should be considered in any treatment program during a die-off. A large proportion of waterfowl affected with botulism will recover if given simple treatment including access to food and water, protection from predators, and gavage with freshwater for severely affected individuals (Hunter and Clark 1971). Administration of 75 or more IU of antitoxin is beneficial; with about 85% of ducks given antitoxin surviving as compared with about 65% of ducks that did not receive antitoxin (W. Jensen 1980, personal communication). In evaluating the value of treatment, such as in botulism and oil contamination, success has usually been calculated based on the proportion of individuals that survived until they could be released.

This may not be an adequate indicator of the success of these individuals in rejoining the population. The long-term survival of treated animals seldom has been evaluated. Any treatment program of this type is labor-intensive and most of the costs are associated with personnel. Treatment of birds in oils pills has been done largely by dedicated volunteers, and the same probably could be done in outbreaks of avian botulism. By using volunteers, costs can be reduced, and a large number of individuals can feel that they are contributing to the solution of a problem.

Public reaction is one factor to consider in deciding whether treatment should or should not be attempted in a disease outbreak. The public is concerned about wild animals, so that there may be considerable pressure to do something, even in situations where nothing can or, perhaps, should be done. A decision not to do anything should be accompanied by public education to explain the situation and consideration also should be given to ways in which the public concern and attention generated by the disease occurrence can be channeled into constructive applications, such as habitat acquisition or improvement to prevent further disease occurrences.

Animals may be treated for some purpose other than to have an effect on the population. Rehabilitation clinics for injured or sick wild animals fall in this area. Rehabilitation of injured individuals of common species, such as red-tailed hawks in North America, has no significant effect at the population level. One reason advanced for maintaining rehabilitation facilities for common species is that experience gained in treating abundant species is valuable in developing techniques for dealing with endangered species. Some would argue that since most of the injuries are a result of human activities, it is morally proper to attempt restitution. Based on my observations of veterinary students helping with a rehabilitation center, such rehabilitation projects are beneficial for humans, if not directly for the population of the animal species involved. The experience gained by the volunteers raises their level of awareness of environmental issues and concerns, and provides an experience with wild animals that would otherwise be unavailable to many in our increasingly urban populace. Such programs provide a valuable vehicle for education about the biology of wild animals and of their requirements for environments outside of the hospital cage.

13.1.1 Treatment of individual animals

When one is dealing with a small number of individuals of rare or endangered species, each of which has high value as genetic stock, it is justified to attempt intensive measures. Thus, one would treat an injured or sick whooping crane differently than one might treat a similarly affected sandhill crane. Examples of treatment of individual rare animals include the prophylactic use of antibiotics to protect roan antelope exposed to anthrax (Hugh-Jones and de Vos 2002), treatment of lead poisoning in white-fronted geese in Japan

(Murase et al. 1992), and capture and treatment with antibiotics of endangered kakapo exposed to erysipelas (Gartrell et al. 2005). Individual treatment has also been used in attempts to establish a herds of Spanish ibex free of sarcoptic mange (Leon-Vizcaino et al. 2002) and wood bison free of tuberculosis and brucellosis (Nishi et al. 2001).

A remnant population of desert bighorn sheep in New Mexico was treated intensively for psoroptic mange (Lange 1982). There were 200–250 animals in 1978 when mange was first recognized in hunter-killed rams. The population declined to about 70 animals by 1979 and this was assumed to be a result of epizootic mange. Disease management was attempted initially using bags containing acaricide in inert dust suspended over salt blocks at about 40 locations where sheep occurred. The hope was that sheep would be dusted with acaricide while using the salt, but this was unsuccessful, probably because the sheep were too wary to use the salt. [A similar method has been used to treat deer with topical acaricides to control ticks (Ginsberg and Stafford 2005)]. The next method used was to capture sheep for treatment, using a helicopter and either a net gun or tranquilizer gun. Captured sheep were dipped in Toxaphene solution, held in captivity for 10–14 days, redipped, and then transported to a large holding area in another part of the state. Forty-nine sheep were captured and treated in the fall of 1979; all were infected with mites. Of these, 29 (59%) survived the procedure and were free of mites after treatment. In the spring of 1980, the 20–30 sheep remaining in the wild were treated with injectable ivermectin delivered as a powder in an absorbable "biobullet" fired by airgun from a helicopter. This was repeated in the autumn of 1980 and treated sheep examined in the winter of 1981 were virtually free of mites. Lesions that had been present on some sheep at the time of treatment had regressed. The 25 animals remaining from the group removed for treatment were returned to the original area and released in 1981. The program was judged to have been successful, although it was recognized that the methods used had controlled (but not eradicated) the infection (Crenshaw 1980). The cost to capture and dip individual sheep was about $ 2,000/sheep, while that for delivery of the injectable drug from a helicopter was $ 100–200/animal (Lange 1982). Mange subsequently recurred and by 1983 only 28 sheep could be found in the wild. The control program was then changed to capture of animals with visible lesions for treatment, and it was concluded that continued annual treatment would likely be required (Pederson 1984). The effort and cost for treating these few animals was justified on the basis that they represented the final remnant of the indigenous wild sheep of the area and were in danger of extirpation as a result of the disease.

Two attempts were made to eliminate infestation with the louse *Trichodectes canis* on wolves and coyotes in Alaska, using a combination of capture of individual wolves for treatment with ivermectin and distribution of ivermectin-treated baits. Although treatment "*appeared to rid at least some of the infested animals of lice*" (Golden et al. 1999), treatment was unsuccessful in eliminating the infestation from the population, because of

difficulty in catching and treating all infested animals. Treated baits were of limited value because they only were applied over a small geographic area. Enzootic infestation in domestic dogs was believed to be the initial source of infestation and remained as a reservoir of the parasite.

The efforts to eliminate ectoparasites described above suggest that such attempts are unlikely to be successful unless every infested individual in the population can be treated effectively, and that these individuals can be maintained in an uninfested state until the last affected animals is treated. If these conditions can not be met, reinfestation will occur.

Individual treatment should be used to **reduce** the risk of transplantation of diseases agents whenever it is necessary to move animals from a potentially infected area into an uninfected area. However, treatment should not be considered adequate mitigation for what is at best a bad practice, and **treatment must not be relied upon** as the sole method for preventing transfer of disease agents. Examples such as the introduction of *Elaphostrongylus cervi* into Australia (Presidente 1986) and *Dermacentor albipictus* into New Zealand (Heath 1986) with treated animals illustrate the risk in relying on treatment to eliminate disease-causing agents. Most drugs that might be used in such programs have not been tested adequately in wild species, so that their efficacy is unproven. One cannot rely on extrapolation from domestic animals as an indication of efficacy, e.g., drugs used successfully for treatment of fluke infections in cattle were ineffective against *Fascioloides magna* in deer (Foreyt and Todd 1976). An additional problem is that a drug, while ineffective in ridding the animal of infection, may mask the infection and make it even more difficult to detect. This occurs with some anthelmintics that reduce or stop the shedding of eggs or larvae for a period, but do not kill adult worms.

While treatment of individual animals has limited application in managing disease at the population level, intervention trials in which disease agents are removed or reduced from a segment of the population have proven extremely useful for understanding the effect of disease agents at the population level. Examples in which anthelmintics have been used in this manner include studies of parasites in red grouse (Hudson 1986), Soay sheep (Gulland et al. 1993), snowshoe hares (Murray et al. 1997), reindeer (Albon et al. 2002) and mountain hares (Newey and Thirgood 2004).

13.1.2 Remote delivery of therapeutic agents

Treatment might be used for intensively managed species where it is possible to deliver the chemotherapeutic agents to individual animals in the field without the necessity of capturing and handling the animals. An example of this form of management was the use of anthelmintics and antibiotics to control mortality caused by pneumonia in bighorn sheep. Bighorn sheep populations in much of North America declined dramatically over the past

century and, in many areas, only isolated remnant herds exist. Some of these herds have had a history of repeated population fluctuations, with the number of sheep increasing gradually until there is a dense population on a small area, followed by an epizootic in which animals of all ages succumb to pneumonia. Lungworms (*Protostrongylus* spp.) play a role in the pneumonic process but opportunistic bacteria, particularly *Mannheimia haemolytica* and *Pasteurella trehalosi*, usually are the ultimate cause of death. Parasitic pneumonia may occur in young lambs as a result of transplacental infection with *Protostrongylus stilesi*. In some herds in Colorado, 95% of lambs died over the summer, recruitment was insufficient to replace adult mortality, and the herds had begun to decline in size (Hibler et al. 1977). The long-term management technique of choice in such herds would be to reduce population density, preferably through translocation of animals to unoccupied range. It was impossible to do this in some herds because of human encroachment and lack of suitable alternate habitat. Attempts to reduce the number of snails that serve as intermediate hosts of the parasite failed, so that the only option felt to remain was to attempt treatment of the sheep (Schmidt et al. 1979).

Initially, sheep were captured with drop-net traps and treated with anthelmintics, either by injection or by drenching, to test different drugs and to determine the efficacy of drugs in increasing lamb survival. In these trials, 80% of ewes treated during 1973–74 had lambs that survived the summer, while only 5% of untreated ewes had lambs that survived (Schmidt et al. 1979). The next step was to develop a system for delivering drugs to sheep without the necessity of capturing animals. Ensiled apple pulp, that was highly attractive to the sheep, was used as bait. Sheep were attracted to feeding sites and, once conditioned to the bait, anthelmintic was added. Schmidt et al. (1979) provided guidelines for use of this method, as well as an assessment of its usefulness. This method was effective in reducing lamb mortality in small herds of sheep over the short-term but it did not cure the population of lungworms, nor did it deal with the basic problem of overpopulation on small areas of heavily contaminated range. Anthelmintics were used as a symptomatic treatment to reduce the immediate signs of distress without affecting an overall cure. The resulting increase in sheep population may even have exacerbated the basic problem. Longer-term management should be directed at reducing overcrowding and promoting migratory behavior among the sheep so that they do not spend long periods of time in areas heavily populated by infected snails (Thorne et al. 1982).

The same basic technique and bait were used to deliver antibiotics to groups of sick sheep during an all-age die-off (Feuerstein et al. 1980). It was impossible to evaluate the success of this treatment quantitatively, but the investigators observed marked clinical improvement in animals within groups that received antibiotic. Known mortality in the group was limited, whereas the authors were "*certain that all untreated sheep observed away from the bait stations died*".

The use of this general method of delivering drugs, either prophylactically or therapeutically, might be applicable in other situations, provided that: (i) the total number of animals requiring treatment is relatively small, (ii) a large proportion of the animals can be attracted to a few sites for treatment, (iii) effective drugs and bait are available, and (iv) the cost of the treatment is not prohibitive. The method is generally suited to very intensive management where supplementary feeding is common, and is not applicable in the extensive management systems used for most North American wild species. The potential risk that aggregation of animals at artificial baiting suites may enhance transmission of infectious agents must be weighed against the benefits of treatment.

As a final comment, I believe that the use of therapeutic agents to manage disease in wild animals should be regarded as a short-term or interim solution at best, until the problem can be addressed in some other manner. Treatment is probably best suited for diseases that occur sporadically and it is not well suited for dealing with enzootic diseases.

13.2 Immunization

Development of effective vaccines and mass immunization have had a remarkable effect on many infectious diseases of humans and domestic animals. One disease, smallpox, has been eradicated globally by this means. In contrast, there has been limited use of immunization for diseases of free-ranging wild animals, but success in controlling rabies in carnivores through vaccination has raised hopes that the method will be more generally applicable. Rabies will be discussed in detail, but first it is important to consider some general features related to immunization.

13.2.1 General features of immunization

The purpose of immunization is to render an individual animal resistant to an infectious agent. Resistance may be of three basic forms: (i) immunization may prevent the animal from becoming infected, (ii) immunization may not prevent infection but may prevent or reduce clinical effects, (iii) immunization may not prevent infection but may reduce infectiousness of the individual, and, hence, reduce transmission to others.

It is important to understand which form of resistance a vaccine confers because an individual immunized with some vaccines may become infected and transmit the causative agent to other animals, although not developing clinical disease. Leptospirosis is an example of a disease in which some forms of vaccination prevent clinical manifestations, such as abortion, but do not prevent the development of a renal carrier state and transmission of the disease

to other animals. There are many possible scenarios with different vaccines, e.g., the oral polio vaccine that has been effective in humans does not prevent infection by the causative virus but it protects against paralysis by preventing the spread of the virus to the nervous system and it also limits multiplication of the virus in the intestine, thereby reducing shedding and the probability of transmission. Vaccines against *Brucella abortus* do not prevent infection in bison but reduce intramammary, intrauterine and fetal infection and, hence, affect both the rate of abortion and the probability of transmission (Olsen et al. 2003).

The effect of immunization is often perceived to be an all-or-nothing phenomenon; i.e. the vaccine either provides protection or it does not. However, immunization should be viewed as having a partial rather than an absolute effect for at least two reasons. The first is that no vaccine produces protective immunity in every individual that is vaccinated, even under ideal laboratory conditions. In the field, the proportion of animals that respond effectively is influenced by many factors, including dose and route of exposure, sex, age, nutritional and reproductive state, presence of intercurrent disease, presence of maternally derived antibody, and many other factors. The second feature is that no individual is protected absolutely against any level of exposure to the agent. As an example, although vaccines against *Brucella abortus* are highly protective in cattle, vaccine-induced immunity can be overwhelmed by high exposure to the organism.

The objective of an immunization program in a population may be of two types: (i) to protect individual animals from the disease, or (ii) to reduce transmission of the disease among the population. When vaccination was used in outbreaks of anthrax among roan antelope in Africa (de Vos et al. 1973) and bison in the Northwest Territories of Canada (Choquette et al. 1972), the intent was to protect individual animals rather than to reduce transmission of the agent. The same is true of immunization for tetanus in humans and in the potential use of vaccine as part of treatment of birds with botulism (Martinez and Wobeser 1999). These diseases are not contagious between animals. In contrast, vaccination campaigns against rabies in wild carnivores and classical swine fever in wild boar are designed primarily to reduce transmission of the disease within the population, although they also protect individuals. A vaccine that protects the individual from disease but that allows infection and transmission to occur may be adequate for the first purpose, but would be inappropriate for reducing transmission within a population.

If the objective is to protect individuals, one would wish to protect as many individuals as possible within the population. To reduce transmission it is not necessary to protect every individual but some minimum proportion of the population must be protected to be effective. The objective is to produce 'herd immunity'. The proportion that needs to be protected varies with the population biology of the individual disease and the situation in which the disease is occurring. It is worthwhile recalling the differences between

microparasites and macroparasites. The most important differences, in terms of immunization, are that microparasites (viruses, bacteria, protozoa) usually cause transient infection and induce long-lasting immunity in recovered individuals. Macroparasites (helminths, arthropods) tend to produce persistent infections, with reinfection being common, and with immunity depending on the burden of parasites infecting the host. Using only this basic information, it should be apparent that immunization is more likely to be effective as a disease-management technique for conditions caused by microparasites than for diseases caused by helminths or arthropods. In diseases caused by microparasites, the population can be divided into three groups: susceptible individuals, infected individuals, and recovered individuals that are immune. It is not possible to divide the host population into such simple groups in the case of diseases caused by macroparasites, because both infected and recovered individuals may be susceptible to reinfection and the infectiousness of an individual may depend on the burden of parasites it carries.

An infectious disease can maintain itself in a population only so long as the effective reproductive rate (R_{eff}) of the disease is ≥1, i.e., on average, each infectious individual infects at least one susceptible animal (recall that R_0 refers to a totally susceptible population, while R_{eff} is used in a population in which some individuals are resistant by virtue of immunity). One of the factors that determine R_{eff} is the density of susceptible animals in the population. The objective of immunization is to reduce the proportion of susceptibles in the population. If R_{eff} is large, the proportion of the population that must be immunized to produce a significant effect is also large and, in general, diseases with a high reproductive rate are more difficult to control by immunization than are those with a lower R_{eff}. In general, the proportion "*p*" of the population that must be immunized to eradicate a disease must exceed $1 - 1/R_0$ (Anderson 1982). The nature of the relationship between p and R_0 is presented graphically in Fig. 13.1, to illustrate the large proportion of the population that must be immunized when dealing with diseases that have a high R_0 value. As an example, measles in England and Wales prior to 1968 was estimated to have a R_0 value of from 14 to 18 (each infected individual on average resulted in the infection of 14 to 18 susceptibles). It was estimated that approximately 96% of each cohort would have to be vaccinated with a vaccine that was 100% effective to reduce R_0 to <1, if the disease was to be eradicated eventually. Vaccination of approximately 57% of the members of a cohort only reduced R_0 by about 20% (Anderson and May 1982).

The precise proportion of wild populations that must be immunized to reduce R_{eff} of various diseases to <1 is not known and varies from situation to situation, even within the same species. The proportion of susceptibles in the population is determined partially by the birth rate. Anderson and May (1982) suggested that where the human birth rate is high, extremely high rates of immunization may be required to reduce disease transmission. The

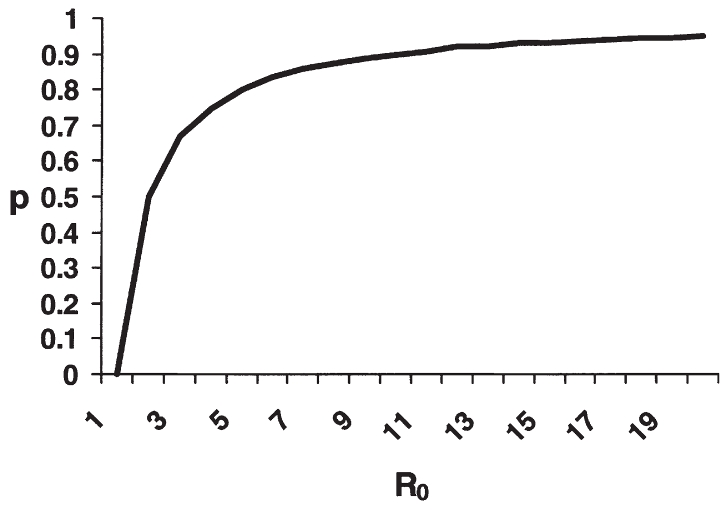

Fig. 13.1 The relationship between p, the proportion of a population that must be immunized to result in the eradication of a disease, and R_0, the intrinsic reproductive rate of the disease. A very large proportion of the population must be immunized in those diseases in which each infectious individual results in infection of many susceptible members of the population

same principle applies to wild animal populations, in that the more rapid the recruitment rate and turnover of the population, the greater the proportion of individuals that are susceptible and must be immunized each season. As an example, assume that we wished to use immunization to control a disease that occurred in both muskrats and beaver. The proportion of the two populations that would require immunization each year likely would be very different. The muskrat population would contain a much larger proportion of susceptible animals than would the beaver population, because of the higher birth rate and more rapid population turnover of muskrats.

The proportion of susceptibles that must be immunized is influenced by the density of animals in the population. This is based on the principle that some minimum density of susceptible animals is required for the disease to remain within the population. If the density of susceptibles can be reduced below that level, the disease should die out. Oral vaccination campaigns have been highly successful in reducing the density of susceptible foxes across large areas of Europe and rabies has been eliminated from these areas. One unexpected event that followed elimination of rabies was that the fox population burgeoned, with densities increasing several-fold in some area. The cause of this increase is unclear and may represent a combination of removal of a major mortality factor (Vos 1995) as well as improvement in the carrying capacity of the habitat (Artois et al. 1997). However, an alarming consequence of the increased population density is that rabies will be more difficult to control should it be reintroduced, because there are now more susceptible animals on the ground.

An effective immunization program may produce protective herd immunity but some susceptible individuals always remain in the population. If the

population density increases and the proportion that can be immunized remains constant, the **absolute** number of susceptible animals present will increase. As a simple illustration, assume that the minimum density of susceptible animals required to support disease X is 0.5/km^2. If the population density of animals (all of which are susceptible) is 0.75/ km^2, an immunization program that protected slightly more than 33% of the population should be sufficient to reduce the density of susceptible animals below the threshold and the disease should not be able to persist. However, if the density is 1.0 animal/km^2, >50% of the population would need to be immunized, and at 2 animals/km^2 at least 75% of the population would need to be protected, to reduce the absolute number of susceptibles below the threshold density. Smith and Wilkinson (2003) modeled the effect of immunization for control of rabies if it should be introduced into the United Kingdom. The critical threshold density of foxes below which rabies would fail to become epizootic was thought to be about 0.2 fox family/km^2. At low fox density (0.25 fox family/km^2), immunization would be likely to control the outbreak, even if only about 20% of the foxes consumed an oral bait containing vaccine. At a density of 1 fox family/km^2, uptake of bait by at least 80% was required to give an 80% probability of eliminating rabies. As stated by Artois et al. (1997) "*to eradicate rabies in a strongly increasing population, one must vaccinate a lot of foxes*".

An important factor in any immunization program is the average age at which individuals are exposed to the disease ('A'). Immunization must occur before exposure if the immunization is to have any significant effect and, thus, the average age of vaccination of individuals must be younger than A. It is difficult to find estimates of A for diseases of wild animals, reflecting the paucity of cohort studies in wild animals, but in some cases it is possible to estimate A indirectly. Hoff et al. (1974) found that 84% of deer >15 months of age in one area of Texas had antibodies to bluetongue virus. The proportion of fawns at 6 and 12 months of age that had antibodies was 36 and 50%, respectively. Transmission of the disease in the area was distinctly seasonal, occurring only during the autumn. Thus, most deer were exposed to the virus during their first autumn and A was between 6 and 12 months of age. To be effective, a vaccination program would have to immunize fawns during the first summer of their life. However, 93% of neonatal fawns in June had antibodies to bluetongue acquired through colostrum from their dam. These so-called maternal antibodies persist for at least 8 weeks and are likely to interfere with the development of immunity following vaccination. Thus, for this disease there would only be a narrow window of time in late summer, following decline of maternal antibodies and before natural exposure to the virus, during which a vaccination program would be effective.

In general, diseases in which animals are exposed at an early age, i.e., those with a low A, will be more difficult to control by immunization than those with a later average age at first exposure. Diseases transmitted vertically from adult to offspring at a very early age are particularly problematic and may not

be amenable to management by immunization. For instance, many European badgers become infected from adults prior to leaving the natal den ('pseudo-vertical transmission') and, thus, are exposed to tuberculosis prior to any opportunity for immunization (Anderson and Trewhalla 1985).

In summary, based on the principles discussed above, it appears that immunization is best suited as a disease-management tool for diseases caused by microparasites that have a small rate of reproduction, and in populations in which the turnover is slow and the average age of exposure to the agent is relatively late.

The basic requirements for a successful immunization program are:

- an effective vaccine
- an effective method of delivering the vaccine to the animal
- the ability to immunize a sufficiently large proportion of the population prior to their exposure to the agent

13.2.2 Vaccine characteristics

Some general features of an effective vaccine are that it:

- produces no significant disease in the host
- stimulates long-lasting (ideally life-long) immunity
- is protective against all varieties of the agent present in the area
- is incapable of reversion to virulence or genetic reassortment with the wild-type agent
- permits differentiation between immunized individuals and individuals that are immune because of infection

The first characteristic is obvious, although in some instances, even in humans, a mild disease reaction to immunization is acceptable. A difficulty in working with wild animals is that their reaction to vaccines is usually unknown and even relatively innocuous material may provoke severe or fatal disease when applied to an untested species. This is particularly troublesome when dealing with rare or endangered species in which there is little opportunity to experiment or test the safety of a vaccine. As an example, inactivated canine distemper vaccines that are safe in domestic dogs and most wild carnivores produced fatal disease in lesser pandas, gray foxes (Bush et al. 1976; Carpenter et al. 1976) and black-footed ferrets (E.T. Thorne, personal communication 1988). Even a vaccine that appears innocuous may have serious consequences under certain conditions. There is a substantial literature documenting the trade-off between mounting an immune response and reproductive success (e.g., Råberg et al. 2000; Ilmonen et al. 2000; Martin et al. 2003) and Hanssen et al. (2004) documented a dramatic decline in overwinter survival of female eiders that mounted an immune response to non-pathogenic antigens inoculated during the nesting season.

Because of the difficulty in delivering vaccine to wild species, it is highly desirable that immunity is established after a single exposure and that protective immunity persists for a long time, reducing the need for booster shots or reimmunization. This has not been the case with some vaccines tried in wildlife, e.g., annual revaccination was required with an anthrax vaccine used in wild bison (Lyster and Stelfox 1977) and with certain early rabies vaccines used in raccoons (Brown et al. 1990).

Many varieties or serologic types of some agents may be present simultaneously, or within a short time period, in a population of animals and a single vaccine may not be protective against all the types. Influenza infections in birds provide an extreme example of this problem. Hinshaw et al. (1980) found 27 distinct influenza viruses in a population of wild ducks on one lake. A similar situation has been detected with bluetongue, in which 19 different strains of virus occurred over time in a cattle herd in Kenya (Davies 1978). In such situations, it is critical to know the types of agent that are present, the most important forms, and the breadth of protection offered by the vaccine. Polyvalent vaccines may be required, e.g., cattle in South Africa were immunized against 15 serotypes of bluetongue virus using three pentavalent vaccines (Schultz and Grieder 1987).

Immunity produced by vaccination should be distinguishable from that produced by infection. This is particularly important for vaccines that protect against disease but do not prevent infection. In such situations it is important to be able to distinguish animals that have antibodies as a result of immunization from animals that have antibodies as a result of infection, because the latter animals may be infected and infectious for others. It may be impossible to evaluate the efficacy of a vaccination program in reducing transmission of a disease if one cannot distinguish the source of immunity. As an example, Strain 19 vaccine for *Brucella abortus* infection in bison induces persistent serologic reactions that interfere with identifying infected animals (Davis et al. 1991), while a second vaccine (RB51) does not induce antibodies that react in standard serological tests for brucellosis (Olsen et al. 2003).

There are many types of vaccine, only some of which have been used in wild animals:

- killed or inactivated vaccines. The agent's ability to enter cells and multiply is destroyed by heating, chemicals, or other means, while its ability to stimulate an immune response is retained. Killed agents are generally safe but may have limited ability to elicit protective immunity. An inactivated canine distemper vaccine has been tested in African wild dogs (Cirone et al. 2004)
- attenuated vaccines. The agent is alive and will enter cells and stimulate an immune response, but has been modified so that it is less pathogenic to the host. Live agents are often highly antigenic but there are potential risks including direct pathogenicity of the vaccine agent to both target

and non-target species, reversion of the attenuated agent to virulence, selection of antigenic variants in the immune population, and genetic reassortment of the vaccine strain with wild agents. Some modified live-virus vaccines used in the field to vaccinate carnivores against rabies have caused vaccine-induced rabies in both target and non-target species (Hanlon et al. 1998). The possibility of genetic reassortment of vaccine and wild genome segments is also a potential risk, and has been reported to occur with bluetongue virus in cattle (Schultz and Grieder 1987)

- similar pathogen vaccine. The vaccine consists of a live agent that is sufficiently similar to the agent that the immunity stimulated is cross-protective, e.g., cowpox virus was used as a vaccine for smallpox in humans
- subunit vaccine. Genetic material related to a small part of the pathogenic agent is inserted into tissue culture cells or plant tissue. The cells produce the subunit which is then harvested from culture and used as a non-living, but highly immunogenic vaccine. A vaccine against anthrax has been produced by inserting a gene from the bacterium into chloroplasts of tobacco plants and then harvesting the antigen for use as vaccine (Koya et al. 2005)
- recombinant vaccine. The most common vaccine of this type involves inserting a gene coding for an antigenic fragment from the disease agent into a virus, and then the live recombinant virus is used as a vaccine. A recombinant vaccine in which the gene for rabies glycoprotien has been inserted into vaccinia virus (V-RG) has been used widely in wild animals in Europe and North America. This vaccine negates the problem of reversion of attenuated rabies virus to pathogenicity; however, there are concerns related to the artificial creation of such novel forms of life and the possibility of infection with the carrier virus in non-target species. An exciting development is the insertion of subunits from disease agents into transgenic plants that can then immunize animals when the plant is ingested, e.g., genes that produce proteins from the bacterium *Escherichia coli* and swine transmissible gastroenteritis virus have been inserted into corn, and corn grain elicited protective immunity when fed to laboratory animals (Streatfield et al. 2001)
- toxoid vaccines. These are designed to stimulate the immune system to combat a toxin produced by the disease agent and usually consist of toxin that has been neutralized so that it is not damaging. A toxoid vaccine has been used to protect wild birds in zoos against botulism (Cambre and Kenney 1993) and its use was suggested as part of treatment for birds with botulism (Martinez and Wobeser 1999)
- Naked-DNA or genetic vaccines consist of a gene from the pathogen that is replicated *in vitro* and which enters host cells and causes production of a protein that is immunogenic. A DNA vaccine has been used in a zoo population of African black-footed penguins to protect against avian malaria (Grim et al. 2004)

13.2.3 Vaccine deployment and delivery

Vaccines can be delivered to wild animals by: (i) direct administration to captured individual, usually by injection, (ii) remote delivery to individual animals without capture and handling, or (iii) remotely through oral baits.

13.2.3.1 Immunization of individual animals

Direct administration is widely used in captive rearing programs for rare species, e.g., eastern loggerhead shrikes were immunized with a West Nile virus vaccine when the disease occurred in a breeding facility (Bertelsen et al. 2004). In some situations it may be practical to capture free-living individual animals for immunization. For instance, Gartrell et al. (2005) captured and immunized endangered kakapos against erysipelas. A more extensive program of this type is trap-vaccinate-release (TVR) that was used to immunize wild carnivores against rabies in the city of Toronto, Canada (Rosatte et al. 1986a; Rosatte 1987). More than 80% of the local population was captured in the first four nights of trapping in an area and 71% of skunks and 90% of raccoons in the area were immunized (Rosatte 1987) at a cost of \$450 to \$1,150/km^2. It was concluded that skunk rabies was controlled successfully by this means (Rosatte et al. 1992) although annual revaccination would likely be required (Rosatte et al. 1990). TVR has been used to create a barrier or cordon sanitaire in advance of the anticipated movement of raccoon rabies into Ontario (Rosatte et al. 1997), to create a ring of immunized raccoons, skunks and cats around the initial cases of raccoon rabies in Ontario (Rosatte et al. 2001), and to control an outbreak of rabies in skunks in Flagstaff, Arizona (Engeman et al. 2003). TVR has been suggested as a local measure to be used in raccoons around zoos or parks to reduce the risk of transmission of canine distemper to captive animals (Paré et al 1999). Corner et al. (2002) used TVR in a 2-year trial to test the efficacy of bacile Calmette-Guérin (BCG) vaccine in preventing tuberculosis in brushtail possums on a 56-ha site in New Zealand. On average, possums were revaccinated every 5 months. There were significantly fewer cases of tuberculosis in the vaccinated group than in an unvaccinated group. TVR has been used in parts of Spain "*for several decades*" in an attempt to "*increase rabbit populations through vaccination*" against myxomatosis and rabbit hemorrhagic disease (Calvete et al. 2004). The efficacy of this program is unknown but increased mortality of wild rabbits in the first week after capture and handling was documented. This was believed to result from "*the stress of handling in addition to the detrimental effects of vaccination*" (Calvete et al. 2004). TVR is best suited for species that are readily captured, resilient to the effects of capture and handling, and located in relatively small geographic areas. Cassirer et al (2001) described a trial in which pregnant bighorn sheep ewes were captured for vaccination with a combination of two vaccines against *Pasteurella* spp. and *Mannheimia hemolytica* in an attempt to improve lamb survival following a

pneumonia epizootic. Survival of lambs born to vaccinated ewes was lower than that of lambs born to unvaccinated ewes. One possible explanation is that passive immunity acquired from the dam may have interfered with development of acquired immunity in the lambs. The authors concluded that vaccinating in this situation may be contraindicated.

The largest immunization campaign for wild animals that involved handling individual animals was an attempt to protect bison in Wood Buffalo National Park, Canada from anthrax. Between 1965 and 1977 almost 28,000 bison were rounded up into corrals with helicopters and processed through chutes for vaccination (Tessaro 1988). Immunity declined rapidly after 6 months and lasted only 9–12 months (Lyster and Stelfox 1977) so that annual revaccination was required for protection. The total cost and the efficacy of this program have never been assessed; however, only one-third of the population was vaccinated in the most successful year of the program. The total number of deaths attributed to anthrax in the area between 1962 and 1987 was estimated at 1,100, which must be balanced against the death of 624 bison as an immediate result of handling for vaccination (Tessaro 1988).

13.2.3.2 Remote delivery of vaccine to individuals without capture

A few attempts have been made to deliver vaccines remotely, such as by the use of a "*coyote-getter*" to explode inactivated rabies vaccine into the mouth of foxes, and a syringe propelled by a steel trap mechanism for injecting rabies vaccine into the leg of foxes (Debbie 1983), but these proved ineffective. de Vos et al. (1973) used a helicopter and dart-gun to vaccinate roan antelope during an anthrax epizootic. This was judged appropriate for protecting a rare species during an emergency. Remote vaccination of possums with BCG using an automatic vaccinator placed in the field to deliver vaccine as an intranasal or intraconjunctival aerosol has been proposed as one method that might be used in New Zealand (Corner et al. 2001; Corner and Buddle 2005). A major program has been conducted in Wyoming for a number of years to immunize elk against brucellosis using freeze-dried modified live bacterial vaccine (Strain 19) delivered in the form of a resorbable biobullet fired from an airgun (Kreeger and Olsen 2002). Between 1985 and 2002, >40,000 elk, primarily calves, were vaccinated during winter when they concentrated on artificial feedgrounds. The efficacy of the program has been assessed by measuring the proportion of animals in the herd that have antibodies to *Brucella* (as a result of infection). Vaccination resulted in a reduction in seroprevalence over several years, and then the rate of seroprevalence increased to a level similar to that in an unvaccinated herd. This was traced to the use in one year of vaccine that had lost potency, so that a cohort of elk was not protected. "*The dramatic increase in seroprevalence after just one year of "missed" vaccination underscores the importance of continued vaccination*" (Kreeger and Olsen 2002). These authors concluded their review of vaccination of elk with

the statement that it is "*probably unlikely, that vaccination will result in eradication of brucellosis in elk*".

13.2.3.3 Oral vaccination

The most promising method for mass immunization of wild animals is through vaccines that are effective when ingested in bait. Vaccination in this manner negates the need for capturing animals, which is only possible under special circumstances and on small areas, it avoids injury and harm that can occur when animals are restrained and handled, and it does not cause the aggregation of animals that may enhance disease transmission. However, oral immunization presents a new range of challenges. The first is that the vaccine must produce immunity by the oral route. Inactivated rabies virus vaccines do not produce satisfactory immunity by this route (Rupprecht et al. 1992) but several attenuated virus vaccines and V-RG produce protective immunity in red foxes when given orally (Blancou et al. 1986, 1988). Attenuated vaccines do not produce immunity in raccoons or skunks by this route (Rosatte 1987; Rupprecht et al. 1990) but V-RG has proven efficacious in raccoons, gray foxes and coyotes and by the end of 2003 almost 50 million baits containing this vaccine had been placed in the field in North America (Slate et al. 2005). No vaccine is available that will produce sufficient population immunity in skunks by the oral route (Slate et al. 2005). A modified live virus vaccine (Kaden et al. 2000) delivered in baits has been used for vaccinating wild boar against classical swine fever as part of eradication efforts in Germany (Kaden et al. 2005).

A second concern is that the vaccine is placed free in the environment rather than into the animal, so there is loss of control over whom or what may have access to the vaccine. This is not a major problem if killed vaccines are used but if live infectious agents are employed there is a risk that: (i) individuals of the target species may consume multiple baits and suffer ill effect, and (ii) that the vaccine may be ingested by non-target species. The vaccine should be non-pathogenic for non-target animals, including humans, which might inadvertently encounter vaccine-laden baits. This was a major concern in the use of attenuated virus oral vaccines for rabies. Some vaccine strains produced disease when given orally to certain species of rodent under laboratory conditions and there have been isolated instances of vaccine-induced rabies in wild animals during field use (Hanlon et al. 1998). There is no risk of vaccine-induced rabies with V-RG but there is a potential for infection by vaccinia virus, and one case of human vaccinia infection after exposure to vaccine has been documented (Rupprecht et al. 2001).

Oral vaccines must be stable and retain immunogenicity for as long as possible under adverse environmental conditions, as there may be a delay before the bait is found and ingested. The degree of stability required is dependent upon how rapidly baits are taken up by the target species and environmental

conditions. For example, the live virus vaccine used in the initial campaign against rabies in foxes in Switzerland retained a critical titre of vaccine virus for at least 3 days at temperatures up to 37°C, which was judged to be adequate, as 63% of baits disappeared within 48 h (Steck et al. 1982). The titre of V-RG used to vaccinate raccoons in New Jersey was maintained near the initial titre for >3 months when enclosed in a bait cylinder (Roscoe et al. 1998). Vaccination using attenuated rabies vaccines is not advised at temperatures below 0°C because frozen vaccine does not induce sufficient immune response and the virus titre may be decreased by freeze–thaw cycles (European Commission 2002). Lyophilized attenuated vaccine might be suitable for use under winter conditions (Follmann et al. 2004). Rabies vaccination programs in Europe have generally been done in spring and autumn to avoid unfavorable conditions in summer but emergency vaccination programs could be done using V-RG that is highly heat-stable (European Commission 2002).

If a suitable vaccine is available, the next requirements are for attractive bait and a method for distributing the baits. Baiting systems are most advanced for rabies vaccination. A variety of baits have been used to delivering rabies vaccine to foxes including chicken heads (Steck et al. 1982), sausage bait (Baer 1985) and meat balls in plastic bags (Johnston and Voigt 1982). The most common form currently in use is a sachet or plastic blister pack containing liquid vaccine enclosed in a wax or polymer material combined with various attractants. Different target species require different baits and there is no universal carnivore bait. A similar sachet type of bait is used for delivering classical swine fever vaccine to wild boars (Kaden et al. 2000).

Baits must be placed at suitable density in appropriate habitat. The methodology has evolved from hand delivery of baits to automated delivery from aircraft supplemented by hand delivery in areas where baits can not be dropped, such as within urban areas and to specific sites such as known fox dens. Game wardens and policemen were the principal method used to hand distribute an average of 15 baits/km^2 in Switzerland (Steck et al. 1982). Approximately 400,000 baits were placed at an average density of 15.5/km^2 in Germany "*almost entirely by private hunters*" (Schneider 1985). Rosatte et al. (1992) placed baits immediately about fox dens and along ravine systems in metropolitan Toronto. Johnston and Voigt (1982) used a low-flying aircraft to drop baits at a density of 35/km^2 in Ontario. Aerial delivery of baits at about 20/km^2, supplemented by limited hand delivery, was used in a multiyear program that eliminated fox rabies from an area of about 30,000 km^2 of Ontario (MacInness et al. 2001). Distribution from helicopters and vehicles was used to distribute rabies vaccine to create an 18-km-wide barrier in advance of a wave of raccoon rabies in New Jersey (Roscoe et al. 1998). In this situation, baits were concentrated in heavily used raccoon habitat at a density of about 250/km^2(overall density about 64/km^2).

The objective of oral immunization is to produce herd immunity. The actual level of immunity required to control or eradicate a disease depends upon the characteristics of the disease, the density and composition of the

host population, the number of baits distributed, the proportion of target animals that ingest baits, and the proportion of animals which ingest baits that become immunized. It is important that the baits are distributed in a manner so that a significant proportion of the population is exposed and the number and density of baits required varies with the density and home range size of the target population. In Europe, 18–20 and 20–30 baits/km^2 were recommended for low and high fox population densities, respectively, and it was stressed that all fox home ranges regardless of shape or size need to receive several baits (European Commission 2002). Markers such as tetracycline may be incorporated in the bait so that animals which consume bait can be identified after the immunization campaign. The proportion of marked foxes in a population, i.e., animals that consumed at least one bait, following baiting has been about 70–80% among foxes in rural areas in Europe (Masson et al. 1999) and about 40% in foxes in urban areas of the United Kingdom (Trewhalla et al. 1991). About 52–67% of wild boars were marked with tetracycline after use of oral vaccine for classical swine fever (Kaden et al. 2000). Not every animal that ingests a bait is immunized, e.g., in one study 84% of raccoons had tetracycline marking while 57% had antibodies to rabies (Hanlon et al. 1998). In another study, tetracycline marking and antibodies to rabies virus glycoprotein were detected in 73 and 61% of raccoons, respectively, following an immunization campaign (Roscoe et al. 1998).

The intensity of baiting campaigns has been highly variable and techniques need to be tailored to the individual situation, with continual monitoring to measure success. To immunize foxes against rabies, a single application of vaccine in the autumn of each year was used in Ontario (MacInnes et al. 2001), while the program in most parts of Europe used applications in spring and fall (Aubert 1994). It has been suggested that other schedules, including summer immunization, might be more cost effective (Selhorst et al. 2001). Oral vaccine for classical swine fever in wild boar was applied much more frequently (two applications separated by a 14 to 28-day interval, repeated at 3 to 4-month intervals throughout the year) (Kaden et al. 2005).

There is sufficient information available from oral vaccination campaigns against rabies in several countries to draw some general conclusions:

- Prevalence of rabies can be reduced relatively quickly through intensive vaccination but elimination of disease over large areas requires consistent application of the program for several years. The oral vaccination program for fox rabies in France began as a 54,792-km^{2}belt across the country in 1989–90 and included the entire enzootic area (192,418 km^2) in 1992. The last recorded case of rabies was in 1998 (Toma 2005). Oral vaccination in the Czech Republic began on a limited scale in 1989 and throughout the entire country in 1993. The last case was recognized in 2002 (Matouch and Vitásek 2005). The elimination of arctic fox strain rabies from eastern Ontario, Canada was accomplished by seven consecutive vaccine-bait campaigns conducted once each year (MacInnes et al. 2001).

- During vaccination programs there should be constant and intensive surveillance of the incidence of rabies, the uptake of bait by target animals (based on tetracycline marking) and the proportion of animals that have immunity.
- Immunization must be done over very large areas (the minimum size suggested in Europe is 5,000 km^2). If an entire infected area cannot be vaccinated as a whole, parts should be treated repeatedly until the rabies is eliminated. There should then be an overlapping area of vaccination in new and previously vaccinated areas to prevent reinfection (European Commission 2002).
- Campaigns should be coordinated across administrative and political borders.
- Any diminution of effort, or inadequate distribution of baits, prior to complete eradication is likely to lead to new outbreaks. Failure to eliminate residual areas of rabies in western Germany was attributed partly to inconsistent distribution of vaccines in areas where baits could not be distributed from the air and insufficient priority being given to rabies control in the final phases of the campaign (Müller et al. 2005). Elimination of rabies in the Czech Republic was complicated, prolonged, and made more costly because limited financial resources made baiting suboptimal in some foci and created unprotected areas within the vaccination zone (Matouch and Vitásek 2005). Roscoe et al. (1998) identified insufficient funding and a decision to discontinue vaccination as factors that contributed to the breach of a vaccination barrier designed to prevent raccoon rabies entering an area of New Jersey.
- Intensity and method of baiting must be tailored to match the density of animals. A problem encountered in parts of Europe has been exceptionally high density and small home range size of foxes in urban areas where baits must be delivered by helicopter or hand. A second problem is that fox populations have increased where vaccination has been successful. The European Commission (2002) recommended that baits be distributed at a density of 18–20/km^2 and 20–30/ km^2 and along flight lines 500 and 300 m apart in areas of low and high fox density, respectively. Extraordinarily high density of raccoons in response to intentional feeding or access to refuse creates similar problems in urban areas in North America.
- When vaccination is used to create a cordon sanitaire between infected and uninfected areas, or around isolated foci of disease, the zone of vaccination must be larger than the maximum distance traveled by the target species. If there are no natural or artificial barriers that limit movement of foxes, the European Commission (2002) recommends a minimum radius of 50 km about isolated foci and a zone 50 km wide as a barrier. Larger distances were recommended if the disease involved raccoon dogs. A vaccination zone 18 km wide slowed but did not prevent the advance of raccoon rabies into an uninfected zone in New Jersey (Roscoe et al. 1998). A zone 65 km wide was established along the Rio Grande River to prevent reinfection of an area in Texas from which canine rabies, primarily in coyotes, had been eliminated (Slate et al. 2005).

- Immunization programs should use natural barriers to advantage. In Switzerland, alpine areas more than 2,000 m in altitude acted as a barrier to the spread of rabies between valleys, so that vaccination zones could be established across valleys in front of an advancing wave of disease. Large rivers act as "*semipermeable barriers*" that slow the advance of raccoon rabies (Smith et al. 2002). The presence of such a barrier may be very important in reducing movement of animals and disease into the vaccinated area.
- If rabies is eliminated from an area, immunization should be continued for at least 2 years after the last case of rabies is reported (European Commission 2002); a barrier zone should be maintained to prevent entry from adjacent areas where the disease still exists; and enhanced surveillance should be done to detect incursions rapidly so that intense control can be applied locally. It was recommended that 8 foxes/100 km^2 should be tested for 2 years after disease elimination (WHO 1992).
- Oral vaccination is expensive. For example, the cost to vaccinate raccoons in a 64,000-km^2 area in the eastern USA in 2003 was \$96/km^2 (Slate et al. 2005). When the prevalence of disease becomes very low or the disease appears to have disappeared there is a real risk that vaccination programs will be stopped too soon, because of the cost (Mackenzie 1997).

Rabies was used as the model for discussion of oral immunization because of the information available on the disease. However, rabies is more amenable to control by immunization than most other diseases of wild animals. Rabies is directly transmitted usually with only one (or at most a few) host(s) important for its maintenance, it has a relatively low R_0, vaccines are available that confer long-lasting immunity to the strains of virus active in the wild, there is no complicating factor of naturally acquired or maternally transferred immunity with rabies, and unvaccinated animals that become infected die and are removed from the population. Many other diseases have a multiplicity of hosts, occur as a variety of strains, have larger R_0, and infected individuals persist in the population. This is particularly true of those diseases that are transmitted indirectly. Immunity to many agents also is transient. In such instances, it may be necessary to maintain almost 100% immunity through continual vaccination campaigns using polyvalent vaccines. The feasibility of such a campaign remains to be tested but has proven to be extremely difficult to accomplish in human populations.

13.3 Summary

– Most infectious diseases of wild animals could be prevented by immunization or treated satisfactorily in the **individual** but few methods are available for delivering therapeutic agents or vaccines to free-living populations.

- Treatment of sick wild animals should be assessed on the basis of significance of the animals that might be saved, probability of success, cost of treatment in comparison to other uses for the money to benefit the species, availability of personnel and facilities, and consequences of not attempting treatment.
- Treatment may be appropriate for endangered species or intensively managed species where therapeutic agents can be delivered directly to the individual or small group.
- Treatment should be used to **reduce the risk** of translocating disease agents when wild animals must be moved but **treatment must not be relied on to prevent transfer of agents**.
- Immunization may be used to protect individual animals or to reduce the rate of disease transmission in a population.
- Some vaccines prevent infection, others allow immunized animals to become infected and infectious but prevent the development of clinical disease, others do not prevent infection but reduce transmission of the disease.
- Immunization is more likely to be effective for the control of diseases caused by microparasites than for diseases caused by macroparasites.
- Immunization is best suited for control of diseases that have a low rate of spread in which the average age of exposure to the agent occurs relatively late in life, and in populations with a low rate of turnover.
- An effective vaccine should produce no significant disease, stimulate long-lasting immunity, protect against a wide range of varieties of the agent, and be incapable of reversion to virulence.
- Immunity produced by vaccination should be distinguishable from that resulting from infection.
- Oral immunization, using baits, appears to hold the greatest promise for use in wild animals. Because such vaccines are distributed in the environment rather than introduced directly into the target animal, they must be non-pathogenic for non-target species and retain their immunogenicity under adverse conditions.
- The proportion of a population that must be immunized for disease control is directly related to the rapidity with which the disease is transmitted and to the population density.
- Immunization programs to eliminate disease are expensive and require a commitment to long-term consistent application over large areas to be successful.

14 Disease management through environmental modification

"*Now, perhaps more than ever, there is a need to incorporate the tools of ecology into the design of programs aimed at preventing and controlling infectious diseases*"

(Smith et al. 2005)

14.1 General considerations

Disease management through manipulation of elements of the environment, other than the causative agent or the population of animals, is based on the concept that disease results from interactions among agent, host, and environment. A population of susceptible animals and a disease agent or risk factor may be present in an area much or all of the time, with disease occurring only when certain environmental factors also are present or in certain individuals within the population who are exposed to the agent to a greater degree or who have reduced resistance because of some factor in the environment. The task of the disease investigator is to identify the specific environmental factors associated with the occurrence of disease. If this can be done, the disease might be managed by ensuring that the specific combination of environmental factors does not occur or occurs less frequently. Disease management in this manner is less direct than management that reduces or removes the host population from the area or that eliminates the causative agent, but the indirect approach may be possible in situations where neither of the more direct actions is feasible, and the effects may be more long-lasting. However, manipulation of environmental factors requires a much better understanding of the ecology of a disease than simple identification of its cause. The more thoroughly the ecology of a disease is understood, the greater the likelihood of discovering one or more points at which management may be accomplished through habitat modification. As stated by Leopold (1939), the "*the very complexity* [of disease mechanisms] *increases the possible points of attack, one of which may some day be used for control measures*". As in all other forms of management, the goal is either to reduce exposure to the causative agent/factor, or to increase the ability of the animal to resist the harmful effects of the agent. Most examples of environmental manipulation for disease control in wild animals have dealt with the former of these.

Environmental modification might be used to reduce or prevent the impact of an existing disease condition, or to prevent the introduction of a new disease into an area. It also might be used to mitigate some impending habitat change in order to reduce the risk of disease associated with the change. Many methods for habitat modification are familiar to wildlife managers, foresters and farmers, and include techniques such as soil cultivation, prescribed burning, manipulation of water movement and levels, induced changes in vegetation and, on occasion, disinfection. These techniques may be used to influence the distribution of animals, to reduce the number and availability of disease agents, or to interrupt transmission of disease.

An important concept in attempting management of disease through the modification of environment is the nidality of many diseases. Often the occurrence of a disease is dependent upon factors only present in some small geographical focus within the general habitat. Nidality applies to both infectious and non-infectious diseases, although the concept was originally reported for infectious conditions. Diseases that have a distinct and recognizable nidus of occurrence, critical for their perpetuation, should be more amenable to control than are diseases that are distributed diffusely in nature. Management efforts can be more intense when applied to a small nidus than when they must be applied diffusely over the entire geographic range of the disease.

Environmental manipulation often involves conflict in land use and value judgments. A habitat might be modified to reduce or prevent disease in one species but the modification may have severe effects on other species or the management may reduce the effect of a disease on a population but negatively impact some other factor such as recruitment. The meningeal worm (*Parelaphostrongylus tenuis*) provides an example of the type of value judgment that may occur. This parasite is widespread in white-tailed deer in eastern North America and it causes little or no clinical illness in this species. However, it causes severe neurologic injury in other cervids. This may be important for moose in areas where the geographic range of *P. tenuis*, white-tailed deer, and moose overlap. The prevalence of *P. tenuis* in an area is related directly to the density of deer (Karns 1967). The prevalence of neurologic disease in moose has also been related to deer density, with higher prevalence of disease occurring in areas with dense deer populations (Gilbert 1974). The density of moose has been found to be inversely related to the prevalence of the parasite in deer in some areas (Saunders 1973), suggesting that *P. tenuis* has a negative effect on moose populations. The parasite cannot maintain itself within moose in the absence of deer and, even in areas where the disease is enzootic in deer, small groups of moose may remain free of the parasite because of spatial and temporal separation between deer and moose (Anderson and Prestwood 1981). Moose and deer have different habitat requirements and moose populations have expanded when deer populations have declined in areas where P. *tenuis* is enzootic. It is likely possible to have either a high deer population or a high moose population, but not both, in such an area and "*attempts to increase both moose and deer populations in an area* [where

P. tenuis occurs] are *probably contradictory*" (Anderson and Prestwood 1981). Thus, the wildlife manager has to make a choice: moose or deer. If a larger moose population is the desired goal in an area where *P. tenuis* occurs, habitat could be modified to encourage moose and to discourage deer, perhaps by allowing forests to mature. This change has occurred naturally in large areas of North America where maturing forest has become more suitable for moose and less suitable for deer. One can predict that logging of this mature forest (a choice that will be made for reasons other than disease management) will create better habitat for deer, more deer and an increased prevalence of 'moose sickness' caused by *P. tenuis*.

As another example, many environmental factors that seem to favor occurrence of botulism among waterfowl, such as the presence of shallow, fertile, organic-rich water, containing abundant invertebrates and vertebrates, and with nesting islands and a low density of scavengers/predators, also are optimal for waterfowl production. The most appropriate habitat management for duckling production and for botulism prevention among molting ducks may be directly contradictory. Management might involve balancing the number of birds produced on the marsh against the number that die there of disease. We have found that mortality among nestling Franklin's gulls on one lake provides the initial substrate to start botulism outbreaks among ducks (Soos and Wobeser 2006). On this marsh it might be necessary to choose between diversity, i.e., having a Franklin's gull colony and managing the marsh to reduce loss of ducks to botulism.

In considering the factors that might influence a disease, it is necessary to include both abiotic and biotic features.

14.2 Abiotic elements

14.2.1 Climate and weather

Some factors such as climate and weather are beyond manipulation, but environmental management decisions made to deal with climate or weather may have an impact on disease. For instance, providing artificial sources of water for wild animals during times of drought may produce foci at which disease occurs. Swift et al. (2000) described the death of at least 45 desert bighorn sheep near artificial water sources (guzzlers), apparently as a result of botulism. The carcasses of lambs that fell into and drowned in the tank provided substrate for growth of *Clostridium botulinum* that subsequently poisoned other sheep. Necrobacillosis (*Fusobacterium necrophorum* infection) has been associated with waterholes in arid regions. The disease occurred annually among deer in California, with the severity in any year being related to weather conditions and size of the deer population (Rosen 1962). Severe outbreaks coincided with high density of both deer and livestock on depleted

range during dry years, when the animals were forced to use the few remaining natural waterholes and watering troughs. Heavy animal use reduced the soil of these sites to "*mud, contaminated by droppings*", creating the site at which animals became infected. Preventive measures proposed were to prevent animals from using certain mudholes in gullies by covering them with brush, to cover other open mudholes by bulldozing or to disinfect these areas, to control overflow at water troughs (and hence reduce the muddy conditions), and to create additional clean water sources dispersed over the range by piping water from seeps or springs to watering troughs (Rosen 1962). These recommendations would also reduce losses to miring of deer in drying reservoirs filled with silt (Bader 1984). Provision of artificial feed to enhance survival during severe winter conditions also may concentrate animals, enhancing contact and promoting transmission of infectious agents.

Although weather cannot be modified to reduce disease, an understanding of the effect of weather on disease can be used to predict when and where disease may occur, for instance, rainfall early in the previous summer explained most of the variation in the number of eggs of the nematode *Trichostrongylus tenuis* passed by red grouse (Moss et al. 1993). The ability to predict disease events based on weather may allow other management procedures to be put in place, such as programs to increase public awareness in advance of an anticipated disease occurrence. For instance, a chain reaction has been observed in which increased production of acorns by oak trees leads to increased mouse populations and increased density of larval and nymphal ticks involved in the transmission of Lyme disease (*Borrelia burgdorferi* infection) (Jones et al. 1998). The greatest risk to humans occurs 2 years after an abundant acorn crop, reflecting the June moisture index 2 years earlier (Subak 2002; Ostfeld et al. 2006). Similarly, extended spring drought in Florida concentrates vector mosquitoes and wild birds in small densely vegetated "*refuges*" providing "*an ideal environment for the rapid epizootic amplification*" of St. Louis encephalitis virus (Shaman et al. 2002). When the drought ends, infected mosquitoes and birds disperse and this may lead to a human epidemic. In both of these instances, weather conditions could provide an early warning of a potential public health concern.

14.2.2 Topography and soils

In general, topography cannot be modified for disease management but managers can take advantage of topographical features such as rivers, lakes, and mountain ranges as barriers or natural cordon sanitaire to facilitate other types of management such as vaccination or population reduction. Soils and bedrock may influence disease in many ways and an understanding of this may be useful in determining where disease management may be necessary. For example, presence of *Ixodes scapularis*, a tick that transmits Lyme disease, is associated with sandy or loam soils overlying sedimentary bedrock,

while its absence is associated with acidic clay soils over Precambrian bedrock (Guerra et al. 2002).

There is increasing recognition that a deficiency of certain micronutrients from the soil can have effects on wild animals. Some deficiencies may be related to naturally deficient soils, while other situations involve anthropogenic changes, particularly acidification. For instance, calcium deficiency in nestling black terns raised in a restored bog area resulted from a decline in pH from 5.9 to 4.4 because of acid precipitation (Bientema et al. 1997). Such deficiencies can be alleviated by supplementation under experimental conditions, e.g., selenium supplementation to deer (Flueck 1994), calcium supplementation to passerine birds (Tilgar et al. 2002; Mänd and Tilgar 2003) but, as with many other situations in wild animals, the problem lies in delivering supplemental nutrients to large numbers of animals over large areas. Acidification also can have the opposite effect by making harmful elements such as cadmium in the soil more available, as has resulted in cumulative poisoning of badgers (Klok et al. 2000). In situations where the problem is related to acidification, liming of soils might be used on limited areas but it is probably not feasible over large areas unless it also has value for agricultural crops.

Cultivation of soil has been used to make lead shot less available to birds in both upland (Esslinger and Klimstra 1983) and marsh sites (Fredrickson et al. 1977; Windingstad and Hinds 1987). Similarly, cultivation was proposed to bury waste soybeans that caused esophageal impaction in geese during dry years (Jarvis 1976) and tillage, to bury moldy peanuts, reduced mortality due to mycotoxicosis among sandhill cranes (Windingstad et al. 1989).

14.2.3 Water

Water influences almost every disease of wild animals in some manner through its effects on animal and plant distribution, survival of infectious agents, and as a carrier for disease agents and harmful substances. Four types of water-related illness have been identified in humans (Craun 1986): (i) water-borne diseases, i.e., disease transmitted through ingestion (or inhalation) of contaminated water, (ii) water-washed diseases that are related to poor hygiene and the lack of availability of water for washing, (iii) water-based diseases in which the causative agent spends an essential part of its life cycle in water or in aquatic organisms, and (iv) water-vectored diseases that are transmitted by arthropods that breed in water or bite near water. Of these, all but water-washed diseases occur in wild animals. Anthrax is an example of a disease that may be water-borne. The occurrence of anthrax in African ungulates is strongly associated with the congregation of animals about waterbodies during the dry season (Pienaar 1967; Prins and Weyerhauser 1987). Pienaar (1967) recognized this association and directed management at these foci including fencing to exclude animals, harassment to prevent use

of waterholes by vultures that were thought to contaminate the water by bathing after feeding on animals dead of anthrax, and disinfection of the water using various chemicals. The efficacy of these techniques in controlling outbreaks is speculative, but Pienaar concluded that the results of disinfection "*were too consistent to gainsay their value in such operations*". Prins and Weyerhauser (1987) were unable to use disinfection of waterholes during an outbreak of anthrax in Tanzania because of its cost.

Prevalence of water-borne disease of humans has declined dramatically in the developed world as a result of filtration, disinfection and protection of water sources from human waste and toxic chemicals. None of these protective factors have been applied to water used by wild animals and the quality of water available for wildlife often is very poor. One example is the entry of subsurface drainwater from crop irrigation into wetlands used by aquatic birds. About 200 refuges and management areas in the arid portions of the western USA receive such water (Paveglio et al. 1997). The most serious problem resulting from this water is selenium intoxication of birds (Ohlendorf 1996). Mitigation measures that have been attempted include draining and filling wetlands, replacement of drainwater with freshwater, and creation of freshwater wetlands near saline evaporation basins to dilute the daily intake of selenium by waterbirds. None of these has been completely successful. Drainage and filling results in a safer environment but toxic levels of selenium may still occur in the food chain under certain circumstances (Wu et al. 1995). After 9 years of replacement of drainwater with freshwater, the concentration of selenium in some birds in an area of California remained above the level associated with reproductive impairment (Paveglio et al. 1997). Creation of a freshwater wetland adjacent to a contaminated evaporation basin diluted the dietary selenium exposure in American avocets at one site in California but did not reduce selenium intake by black-necked stilts that continued to nest on the evaporation basin (Gordus 1999).

Other disease situations such as contamination of coastal waters with *Toxoplasma gondii* and *Salmonella* from freshwater runoff and sewage discharge (Bowater et al. 2003; Fenwick et al. 2004) and contamination of wetlands with *Pasteurella multocida*, that will be discussed later, are potentially even more difficult to manage.

Skrjabin (1970) reported managing watering sites to control a water-based parasitic disease he called "*parafasciolopsosis*", [probably infection with the trematode *Parafasciolopsis fasciolaemorpha*, which may be lethal to moose (Soulsby 1982)]. The intermediate host of this parasite is the snail *Planorbis corneus*. Severe infections occurred in moose during dry years when "*moose are forced to concentrate near floodlands of rivers and creeks, these habitats are usually densely populated by the above-mentioned mollusk*" (Skrjabin 1970). To prevent occurrence of the disease, small artificial reservoirs were created within peat bogs by bulldozing. Because of the acidity of soil and water in the bogs, these sites were free of the snail. In one dry year, six of seven moose using the artificial watering sites regularly were found to be free

of the parasite and the seventh animal had "*several*" parasites. In contrast, 17 of 18 moose sampled from areas distant from the artificial reservoirs were infected, with an average burden of 630 flukes per moose. It was proposed that construction of one or two such reservoirs per 1,000–1,500 ha of moose habitat would be adequate for disease management. The extent to which this procedure has been applied is unknown. Infection with the parasitic nematode *Eustrongylides ignotus* has been identified as a major mortality factor in nestling wading birds in Florida (Spalding et al. 1993). Wetlands enriched by nutrient pollutants have very high populations of the oligochaete that is the first intermediate host of this parasite, and fish infected with larvae are highly vulnerable to predation. Coyner et al. (2002) suggested that ponds could be monitored during the breeding season of wading birds and ponds with heavily infected fish could be drained (the beneficial effect of reduced disease would have to be weighed against potential negative effects of reduced food availability for the birds).

Drainage of wetlands has been used successfully for many years to control mosquito-borne diseases of humans, but this obviously also has many negative environmental effects and, to my knowledge, has not been done with the aim of reducing disease in a wild species. Much smaller and more local water management activities may have an impact on disease, e.g., artificial water containers on pastureland and in urban areas have been associated with significantly increased mosquito density compared to ground pools in forest (Leisnham et al. 2005). Management might consist of reducing the number of such sites by removal of artificial containers and the filling of tree holes with concrete as has been done to remove breeding sites used by the mosquito vector of La Crosse virus (Monath 1984). This type of action might be applicable for local disease problem in endangered species such as reducing transmission of malaria to certain species of Hawaiian birds.

14.3 Biotic factors

14.3.1 Vegetation

Habitat change often is most evident as changes in the vegetational components of the landscape and is sometimes associated with a change in disease. For example, invasion of waterbodies by water hyacinth (*Eichornia crassipes*) has been associated with increased snail populations and schistosomiasis in humans (Plummer 2005), and avian vacuolar myelinopathy is associated with invasion of wetlands by *Hydrilla* spp. (Lewis-Weise et al. 2004; Rocke et al. 2005; Wilde et al. 2005). "*Habitat loss is probably the most important factor causing species declines worldwide*" (Sih et al. 2000) but there have been relatively few studies of the effect of habitat loss on disease in wild animals. Without a firm understanding of how habitat change and loss affect disease,

it is difficult to develop appropriate management strategies. Habitat loss may involve: (i) reduction in habitat area, (ii) habitat fragmentation, (iii) habitat deterioration within patches, and (iv) deterioration of the matrix between patches (Sih et al. 2000). Each of these may influence the occurrence and severity of disease.

14.3.1.1 Reduction in habitat area

One potential effect of reduced habitat area is that animals may be at increased density "*as they crowd into the remaining suitable habitat*" (MacCallum and Dobson 2002), increasing the rate of contact and the potential for transmission of infectious agents. This is likely to be a transient phenomenon if the animals occupy the reduced area of habitat on a year round basis because the population size will probably decline through density dependent mechanisms to match the habitat available. However, if the reduction involves only one part of the habitat used by the species, crowding on that portion may continue for an extended period. Loss of wetland habitat previously used by wintering and migrating waterfowl has resulted in birds being crowded on remnant refuge areas for decades. The birds have abundant food available off refuge in the form of agricultural crops, but use of the same wetlands for extended periods allows accumulation of *Pasteurella multocida* (and probably other disease agents) in the water. This appears to be a factor in the emergence of avian cholera as an important disease (Wobeser 1992). In this example, there is no easy management solution for the problem unless new habitat could be created so that the birds can move among wetlands more frequently, as they did in the past, or other measures are taken to reduce bird density.

14.3.1.2 Habitat fragmentation

Fragmentation of habitat usually results from human activities that reduce the size of patches of habitat, increase the distance between patches, and increase the ratio of edge to interior within patches. Under some circumstances, fragmentation of habitat might "*quarantine*" infected patches and reduce the impact of host-specific infectious diseases on the population as a whole (MacCallum and Dobson 2002). Hess (1996) considered the effect of habitat fragmentation on infectious disease and proposed that management to increase movement and interchange among patches, e.g., by establishment of habitat corridors, while beneficial for conservation purposes might increase disease transmission. On the basis of modeling, MacCallum and Dobson (2002) concluded that benefits of corridors outweighed the disease risks and that "*pathogen transmission should not be a critical factor in deciding whether to maintain corridors between habitat patches*".

Planting of coniferous forest that connects fragments of forest in northern England to those in southern Scotland has resulted in genetic mixing of red

squirrel populations that is considered to be beneficial (Hale et al. 2001) but may allow squirrel parapoxvirus to move into Scotland where it has not been recognized (Tompkins et al. 2003).

Reduced biodiversity is a consequence of fragmentation in many situations; however, some species (habitat generalists and species with high population density and small home range size) thrive in fragmented landscapes (Allan et al. 2003). Lyme disease illustrates how these two factors (reduced biodiversity and increased density of a generalist species) may influence an important disease. In eastern North America, *Borrelia burgdorferi* is transmitted by the tick *Ixodes scapularis*, which has four developmental stages during its 2-year life cycle. After hatching from the egg, the larval tick (which is free of *B. burgdorferi*) takes a blood meal from a small mammal or bird. Larvae that feed on an infected animal may acquire the bacterium and remain infected for the rest of their life. Larvae drop off the host after feeding and molt to the nymphal stage, which overwinters in the environment. The following spring or summer the nymph seeks a new host for a blood meal and, after feeding, drops off and molts to become adult. The adult seeks a host (often a white-tailed deer) in the autumn of the second year, takes a blood meal and mates on this host, and then drops off and overwinters before eggs are laid the following spring. Larval ticks are not specific in choosing a host and may feed on a variety of small mammals, birds and reptiles. Most of these hosts are incompetent reservoirs of *B. burgdorferi* and do not infect the tick. The white-footed mouse is a very suitable reservoir (Mather 1993) and most larvae that feed on an infected mouse become infected and pass the infection on to the nymphal stage; the stage responsible for most human infections with Lyme disease (Ostfeld and Keesing 2000). The white-footed mouse is a generalist that often occurs at higher density in fragmented landscapes than in continuous forest (Ostfeld and Keesing 2000). A high diversity of hosts that might be fed on by larval ticks has a "*dilution effect*" in that many larvae will feed on non-competent reservoirs of *B. burgdorferi*, resulting in a lower prevalence of infected nymphs (Ostfeld and Keesing 2000). Thus, fragmentation of forest habitat has two effects: increased population density of white-footed mice and decreased diversity of alternate hosts that might be fed on by larvae. Allan et al. (2003) found "*a dramatic increase in the density of infected nymphs, and therefore in Lyme disease risk, with decreasing forest patch size*". Management in this case probably lies in recognition of the expected impact of forest fragmentation on Lyme disease. This may be used as one argument for protecting larger areas of habitat, as well for providing other protective measures to humans who live in areas of fragmented habitat.

14.3.1.3 Habitat deterioration within patches

Large-scale changes in habitat, such as those caused by agriculture, may have significant effects on the occurrence of disease in wild animals. Stott and Wright (2004) described the occurrence of a high prevalence of abnormalities

of the female reproductive tract in European hares in Australia. The prevalence of abnormalities, which were sufficiently severe to cause infertility, was 46 and 26% in adult hares from two agricultural areas, while none of the 19 hares from an area with natural vegetation had similar lesions. The reproductive lesions were thought to result from exposure to exogenous estrogens that might include phytoestrogens and mycoestrogens from leguminous crops, and estrogenic activity in agricultural chemicals. There would seem to be no easy management solution to this situation because of the relative importance placed on agricultural crops compared to that given to a reproductive disorder in a wild species.

Widespread habitat change may influence disease through increasing the population density of some wild species, often a rodent. For example, conversion of cultivated fields and forests to permanent grassland for dairy production in Europe has had a major impact on the density and occurrence of outbreaks of common and water voles that are intermediate hosts for the zoonotic tapeworm *Echinococcus multilocularis*. Foxes (final host for *E. multilocularis*) "*become specialists and feed almost exclusively on grassland rodents*" in areas with high rodent populations for long periods of time (Giraudoux et al. 2003). Areas of France where rodent population outbreaks last the longest had the largest number of human cases of *E. multilocularis* infection. Changes in farming practices in Australia, including increased diversity and asynchrony of crops (that provide abundant food over an extended period) and clearing of remnant native vegetation that reduces the abundance of predators, contribute to increased problems with outbreaks of house mice in Australia (Pech et al. 2003). Similarly, changes in agricultural practice in Bulgaria led to increases in rodent populations that were associated with tularemia in humans (Kantardjiev et al. 2006). In the situations described above, environmental manipulation to reduce rodent populations seems unlikely, unless it is linked with some agricultural advantage. Overgrazing by livestock is the major factor responsible for serious rodent infestations in Chinese grasslands (Zhang et al. 2003). Species such as Brandt's vole that thrive in short sparse grass are involved in many zoonoses, including salmonellosis, plague, tularemia and tick-borne rickettsial infections. Management by control of grazing pressure resulted in a 78% decrease in vole population density (Zhang et al. 2003) indicating that disease control and agricultural production can both benefit from some forms of management.

'Human-adapted' wild species may flourish under urban or suburban conditions, e.g., the density of raccoon populations is greater in urban/suburban areas than in undisturbed rural areas (Logiudice 2003), at least partially because of availability of clumped resources such as human refuse (Wright and Gompper 2005). This may have disease implications both for raccoons and for other species susceptible to diseases carried by raccoons. Prevalence of endoparasites (including *Baylisascaris procyonis*) increased in raccoons using clumped food resources (Wright and Gompper 2005). Raccoons defecate in latrines to which rodents are attracted by undigested seeds in the feces.

Baylisascaris procyonis is highly pathogenic to intermediate hosts that consume eggs from raccoon feces. The Allegheny woodrat, a rare mammal in eastern North America, is particularly vulnerable to *B. procyonis*. Woodrats introduced into areas with a high density of raccoon latrines had significantly shorter survival time than did woodrats placed in areas with fewer raccoon latrines (Logiudice 2003). Understanding the effects of raccoon density may be helpful for conservation of the woodrat, such as in choosing areas for relocation.

Foxes have adapted to living in urban and suburban areas in Western Europe and Japan. This complicates immunization programs for rabies (see Chap. 13) and has raised concerns that a much larger portion of the human population than previously assumed to be the case may be exposed *E. multilocularis,* leading to "*a major change in the epidemiology of this disease*" (Vuitton et al. 2003). Some raptors have also adapted to urban environments and this may lead to increased exposure to disease agents such as *Trichomonas gallinae* carried by urban feral pigeons. Evidence of the impact of trichomoniasis on urban raptors is inconclusive. In Tucson, Arizona, 85% of nestling Cooper's hawks were infected and about 40% of nestlings died of trichomoniasis prior to fledging (Boal et al. 1998), whereas 9% of nestlings from outside the urban area were infected and none died of the disease (Boal et al. 1998; Boal and Mannan 1999). In contrast, Rosenfield et al. (2002) found a very low prevalence of infection in nestling Cooper's hawks in urban and rural areas and, although about 65% of urban nestling northern goshawks in Berlin were infected, <1% died of the disease (Krone et al. 2005).

14.3.1.4 Deterioration of the matrix between patches.

Although wild species may continue to occupy patches of native habitat, changes in the area between patches may influence the occurrence of disease. This situation may occur where the space between habitat patches has been modified for use by domestic animals that are reservoir hosts for a disease agent that has a serious impact on wild species inhabiting the patches. The endangered Andean deer (huemul) is confined to small reserves in Chile that may be inadequate for its long-term survival (Simonetti 1995). The larval stage of the tapeworm *Taenia hydatigena* is very pathogenic for huemul and causes death under experimental conditions. This parasite cycles between livestock and domestic dogs with minimal effect on either and is present in the area surrounding reserves. The dilemma is that if deer are restricted to protected areas (patches), local extinction is likely as a result of "*demographic vagrancies*", while if they use surrounding areas containing livestock and dogs they may *"face a high probability of death due to increased parasitism"* (Simonetti 1995). A similar situation may exist in parts of western North America where patches of habitat suitable for bighorn sheep are separated by intervening areas in which domestic livestock occur. Contact between domestic sheep and goats frequently has been associated with die-offs of wild sheep

(Foreyt et al. 1994). Singer (1995) reviewed 115 attempts to transplant bighorn sheep in six Rocky Mountain states in the USA. Sheep persisted in 64% of transplants located >32 km from domestic sheep, compared with 44% of transplants located 16–32 km from domestic sheep. It appears that management in this situation must be through regulation to maintain wide buffer zones between wild and domestic sheep.

14.3.1.5 Other forms of vegetation management

One suggested benefit of prescribed burning of vegetation is sanitation and destruction of disease agents. Fire was used as part of the control program for anthrax in bison in northern Canada (Novakowski et al. 1963) and in African wildlife (Pienaar 1967), and has been proposed as a method of destroying lungworm larvae (Anderson and Prestwood 1981). Burning reduced the number of ticks parasitizing young wild turkeys under experimental conditions (Jacobson and Hurst 1979). Ginsberg and Stafford (2005) reviewed the effects of controlled burn on ticks and found that in many situations the reduction in tick numbers was temporary. Boggs et al. (1991) analyzed the effect of prescribed burning and herbicide treatment of pastures on alimentary parasites of cotton rats. The results were complex with some parasites increasing and others decreasing. It is difficult to interpret the results because the effect of treatment on rat density was not reported; however, the authors concluded that: "*man-induced habitat modifications can alter host-parasite relationships*". The efficacy of fire as a disease control measure in other diseases and in field situations is unproven. Pienaar (1967) used fire in managing anthrax. Vegetation was burned in areas affected by anthrax with the primary aim of destroying the bacteria and to facilitate locating carcasses. Burning was also thought to be beneficial in limiting the spread of the disease to new areas as the regrowth that soon appeared after burning "*kept the animals in these areas, and in doing so probably prevented to some extent the straying of affected animals into unaffected areas*".

Habitat manipulation may be used to interrupt or interfere with disease transmission. In 1985, I was asked to advise on management of avian cholera among nesting common eiders on an island (Ile Blanche) in the St. Lawrence River. The island had a history of repeated epizootics dating back to 1964 (Reed and Cousineau 1967). In 1984 the island had about 4,300 nesting eiders, of which approximately 1,000 females died. In 1985 there were about 2,700 nests, 860 eider carcasses were collected, and the estimated loss was >2,000 birds (J. Bedard 1985, personal communication). We compared this island (during the epizootic) to two adjacent islands that also had nesting eiders but which had no history of disease outbreaks. Ile Blanche differed from the other islands in several respects. The most obvious difference was that Ile Blanche had a dense, and almost impenetrable, cover of tall shrubs (*Sambucus* sp.), so

that the surface was shaded and damp, with virtually no air movement at ground level. On the other islands, the shrub/grass cover was shorter, less dense, and incomplete, so that much of the surface was sunlit, dry and windswept. The organic soil on Ile Blanche was poorly drained with numerous, shallow (few centimeters), pools of freshwater. The other islands had gravel or rock substrate and were well drained. *Pasteurella multocida*, the causative agent of avian cholera, was isolated from 4 of 5 samples of surface water collected from pools on Ile Blanche. Gulls dead of avian cholera were found on both Ile Blanche and one of the other islands; dead eiders were extremely abundant on Ile Blanche but none was found on either of the other islands (my suspicion was that gulls on both islands had become infected through scavenging on dead eiders on Ile Blanche).

Based on the comparison, I concluded that conditions on Ile Blanche, particularly the presence of the numerous shaded pools of freshwater, were suitable for in vitro survival of *P. multocida*, whereas conditions on the other islands were not (this organism has been recovered from surface water for from 3 to 30 days after an outbreak (Rosen 1969; Titche 1979; Price and Brand 1984). The dense cover on Ile Blanche also hindered removal of sick or dead birds by predator/scavengers and the continued presence of carcasses would also contribute to contamination of the environment (Titche 1979; Price and Brand 1984). Eiders likely became infected while walking and splashing through these pools, either through skin abrasions or inhalation of bacteria in aerosols. Whether or not eiders drink from the pools was unknown. I concluded that it would be impossible to eliminate the agent, *P. multocida*, if eiders were to continue to occur on the island, but that transmission might be reduced by habitat modification. The suggested management was to drain the surface ponds, remove the dense vegetation, and to establish a vegetation type similar to that on the other islands. The shrub cover was removed by bulldozer and burning, and drainage ditches were dug in the autumn of 1985. Because of the lack of cover, 450 plywood nesting structures were supplied in 1986 and these were used by an average of 1.9 female eiders/ structure. The nesting population in 1986 was about 2,400–2,500 females and no dead eiders were found. Grass was seeded in the autumn of 1986. In 1987, approximately 2,300 nests were counted and 18 birds were found dead (the cause of death of these birds was not determined) (J. Bedard 1987, personal communication). At a later date, spruce trees were planted on the island. No major mortality was reported until 2002, when a major outbreak occurred (Dallaire and Giroux 2005). Because there were no control sites without management, it is not possible to know whether the apparent absence of the disease for 17 years can be attributed to the habitat change.

Habitat modification may be used to influence the distribution of animals in relation to the distribution of disease. Animals may be discouraged from using high risk areas by direct actions, such as draining of wetlands, as has been done to move birds from the site of botulism, avian cholera and lead poisoning outbreaks, or through bush clearing, as was done in conjunction

with game depopulation for control of trypanosomiasis in Africa. A much less dramatic example is the annual pruning of trees in small areas to discourage their use as roosting sites by birds, as a measure to reduce the risk of histoplasmosis for humans (Weeks 1984). Removal of old abandoned farm buildings used extensively as denning sites by skunks might be part of management to control rabies in the prairies of Canada. Animals may also be encouraged to use areas distant from recognized risk factors through creation of attractive alternative habitat. For example, Parrish and Hunter (1969) created new habitat by flooding areas to move ducks away from the site of a botulism outbreak. Rosen and Bischoff (1953) used artificial feeding for a similar purpose. Ivanova (1970) suggested that lungworm infections in wild boars could be controlled by reducing the number of earthworms (the intermediate host of the parasite) eaten by boars. Earthworms were ingested by boars while feeding in particular habitat types, so to reduce earthworm consumption "*intensive summer feeding*" with high protein feeds was used in other areas to encourage the boars to use habitat types that had few earthworms. Dorney (1963) suggested that "*habitat manipulation to encourage segregation of cottontails into distinct summer and winter ranges*" would be helpful in reducing helminth infections, and suggested methods for doing this. Thorne et al. (1982) indicated that habitat management should be used in the control of *Protostrongylus* spp. lungworms in bighorn sheep to ensure that "*herds remain migratory and do not spend too much time on portions of their range that have high fecal contamination, high snail populations, and that are subject to extensive grazing*". In all of the above examples, the intent of the management was to move animals away from areas where they would be exposed to a disease agent.

14.3.2 Animals

Other animal species are a critical component of the environment that can influence the occurrence of disease in many different ways. The most obvious form of interaction is when one or more other species act as host for an infectious disease of the target species. If these other species are maintenance or spillover hosts, i.e. hosts in which the agent can multiply, the effect is to increase the amount of infective agent in the area and the probability of infection of the target species. This is particularly important when the population of the target species is small and the disease agent is maintained in one or more other abundant species. For instance, *Mycoplasma conjunctivae* and *Mannheimia haemolytica* appear to be agents that can be maintained in domestic sheep and spillover into wild chamois and sheep, respectively (Giacometti et al. 2002; Rudolph et al. 2003; Turner et al. 2004). In these cases, effective management of wild sheep and chamois must include restriction of contact with domestic ruminants to prevent disease transfer. Mountain hares do not act as amplifiers for louping ill virus but, because they are additional

hosts for the ticks that carry the virus, the hares allow both the ticks and the virus to persist in red grouse populations (Hudson et al. 1995). If it became necessary to manage this disease, one method might be to manage the hare population. Conversely, if the alternate species is a dead-end host or is refractory to infection, presence of these hosts may reduce disease occurrence in the target species through a diluting effect, as described earlier for ticks and *Borrelia burgdorferi* (Ostfeld and Keesing 2000). Managing habitat to maximize the diversity of species to act as hosts may be helpful in these situations.

Animals that are coinhabitants or that act as competitors, scavengers, prey or predators all may influence disease in the target species, but relatively little consideration has been given to how species that are not directly involved in the transmission of infectious agents might be manipulated as part of disease management. Competitors for resources might place diseased individuals of the target species at an additional disadvantage and exacerbate the effects of disease. Parasites and disease agents can also be a method by which species may compete, i.e., one species may gain a competitive advantage by harboring and transmitting a shared agent to a more vulnerable species (Hudson and Greenman 1998). This may be the case in Britain where the cecal nematode *Heterakis gallinarum* is shared by pheasants and grey partridge. The pheasant is a maintenance host in which the parasite causes little ill effect, while the partridge is a spillover host that is affected severely. The parasite may have contributed to the decline and exclusion of partridge form areas with pheasants (Tompkins et al. 2000, 2001). Management in this case may have to include a decision as to which of these introduced game species is more desirable. Competition and disease may act in an additive or synergistic manner. Parapoxvirus infection in combination with competition from introduced grey squirrels results in a much more rapid decline in red squirrel populations in England than can be explained by competition alone (Tompkins et al. 2003).

Scavengers are likely beneficial in many diseases if they remove infectious or toxic material and do not transfer it to new areas. For instance, scavengers that remove dead birds from wetlands, prior to the development of botulinum toxin in the carcass, reduce the likelihood of botulism occurring (Wobeser 1997) (carcass collection and disposal by workers in airboats is really just an extension of normal scavenging). Similarly, scavengers that remove fetuses or placental material aborted by animals with brucellosis (Cook et al. 2004) might reduce the likelihood of transmission of that disease. However, if scavengers become infected by the agent, as occurs with crows, gulls and raptors that feed on waterfowl dead of avian cholera, or if they carry infectious material on their exterior, as occurs with vultures feeding on animals dead of anthrax (Pienaar 1967), they might transmit the disease to new areas.

Management of a prey species, the earthworm, has been suggested as an alternative to culling badgers for management of bovine tuberculosis in Great Britain. Kruuk (2006) reasoned that because: (i) earthworms are the major prey of badgers, (ii) badger density correlates with the density of earthworms,

(iii) the biomass of earthworm on pastures is enormous, and (iv) badgers require short-grass pasture closely cropped by livestock to capture earthworms, current farming practices lead to an abnormally high density of badgers and short-grass pastures are the "*ideal arena*" for contact between badgers and cattle. Kruuk suggested that earthworm numbers in pastures might be reduced by practices such as increased plowing, grazing rotation that did not result in short-grass sward, or even use of vermicides. An alternative strategy might be to use repellents to deter badgers from feeding in these areas (Baker et al. 2005).

The relationship between predation and disease is complex and multifactorial. In some situations, predatory animals may remove alternate host or vectors of a disease and reduce transmission to the target species. Stauffer et al (2006) described a linkage between the prevalence of schistosomiasis in humans and the abundance of molluscivorous fish in a lake in Africa. The fish prey on the snail that is the intermediate host of the parasite. When the fish population was reduced by overfishing, prevalence of schistosomiasis in humans increased. Conversely, the abundance of snails and the prevalence of human infection decreased as the number of snail-eating fish increased. A direction for disease management is obvious in this case. Animals affected by many diseases have increased vulnerability to predators (e.g., Ives and Murray 1997; Packer et al. 2003) and predators preferentially select diseased animals (Moore 2002), so that predation is the proximate or final cause of mortality of many diseased animals. Superficially it would appear that the effects of predation might be additive to those of disease. However, if predators preferentially remove the most infectious individuals in the population, and if the predator plays no role in transmission of the disease, elimination of the heavily infected individuals (i.e., selective culling) may be beneficial to the target population. Predator control often is used to increase the population of desirable prey species, such as game animals. Packer et al. (2003) modeled the effects of predation on various forms of disease and concluded that predator removal "*can increase the regulatory role of parasites to the point of lowering host population size*" and that "*elimination of low to moderate predation rate is generally harmful to herd health*". The effect of selective culling by predators is likely to be most apparent in diseases caused by macroparasites that are highly aggregated in the prey species. Predators that remove ducks affected by botulism may be the manager's ally, if they reduce the amount of carcass material that will become substrate for further toxin production. Although there is a dearth of empirical evidence, the potential role of predation (beneficial or harmful) on disease should be considered in planning management programs.

14.4 Predicting, preventing, and mitigating

An extremely important part of any program to manage disease in wild animals should be a consideration of the probable effect of proposed or anticipated environmental changes on the occurrence and prevalence of disease.

This might include predicting future risk on the basis of weather or climate changes (e.g., Yates et al. 2002), as well as the probable effect of land use changes on disease. For instance, deforestation and alteration in storage and use of water are likely to provide new niches for disease vectors (Harrus and Baneth 2005), conversion of forest to shrub and grassland will affect rodent communities (Giraudoux et al. 2003), and allowing nutrient-rich pollutants into ponds (Coyner et al. 2002) or sewage to enter the sea (Bowater et al. 2003; Fenwick et al. 2004) are likely to lead to disease in the wild animals that use these areas. Changes in land use, such as conversion for agriculture often result in loss of biodiversity. However, the loss of species is not random. In general, "*the organisms that are losing out have longer lifespans, bigger bodies, poorer dispersal capacities, more specialized resource use, lower reproductive rates...*" (Diaz et al. 2006), while a "*small number of species with the opposite characteristics*", such as some rodents, become dominant. This may have a major impact on diseases for which these species act as a reservoir. Many non-agricultural management activities involve some degree of environmental impact assessment but the potential effect on disease has rarely been a factor in these considerations. However, such consideration is beginning to occur, e.g., resource agencies have begun to include assessment of the probable effect of marsh development projects on the occurrence of botulism in their planning process. In making such assessments, one usually is hampered by incomplete data and may have to consider a number of pieces of inconclusive evidence in order to make the best scientific judgment of the probable effect of any change. Such reasoning with uncertainty is a very common phenomenon in applied biology and is not unique to disease control. We know from accumulated experience that certain types of habitat change or management practice have been associated with disease problems in the past, and one should not be reluctant to use this knowledge for extrapolation and prediction, in the absence of any better information.

In some instances, it may be possible to make very specific predictions, based on prior experience, about the probable outcome of some habitat manipulation. For example, if one were asked for an opinion on probable effects of use of carbofuran for insect control in crops in an area, one could predict that wild birds will be poisoned (Balcomb et al. 1984). Similarly, construction of overhead wires over a marsh will result in bird deaths as a result of collisions and might contribute carcass material to precipitate outbreaks of botulism. In other instances, the evidence is not so conclusive and one can only suggest the possible effect of an environmental change on disease. For example, the giant liver fluke *Fascioloides magna* has a disjunct distribution in North America, probably related to suitable climate and moisture conditions for the snail intermediate host. Irrigation might contribute to the spread of this and other flukes to areas where they had not been reported previously (Foreyt 1981) as has occurred with other flukes that infect humans (Jordan and Webbe 1982; Mas-Coma et al. 2005). This should be considered if widespread irrigation is proposed for an area where suitable ungulate hosts occur and the parasite does not. Similarly, habitat changes such as logging

that favor increased populations of white-tailed deer can be expected to increase the probability of neurologic disease in other cervids in areas where *P. tenuis* occurs.

Any proposed management procedure that concentrates wild animals at high density should be viewed with concern. The occurrence of brucellosis and tuberculosis in cervids aggregated by artificial feeding has been discussed in earlier chapters. We have observed botulism outbreaks among ducks concentrated by artificial feeding to reduce crop depredation. The increased density was probably not an inciting factor but many birds died when the outbreaks occurred, because the feeding program concentrated the birds in an area where toxin was available. The recommendation for developments of this type was that sites be monitored closely and that alternate sites be prepared in advance, so that birds can be dispersed rapidly in the event of a disease outbreak. Friend (1977) described outbreaks of three different diseases in waterfowl concentrated on small areas of water kept from freezing by artificial means during winter. In each case, the density of birds in an unusual location appeared to be a critical factor in the occurrence of the outbreak.

The history of rabies in different countries provides an example of the effect of large-scale habitat changes on disease and the use of prior experience for predicting potential problems. Vampire bat-transmitted rabies in Central America was increased by activities that favored the bats, such as construction of mines, tunnels and wells that provided roosting sites, and livestock ranching that provided an abundant, reliable food base for the bats (Lord 1980). Davis (1974) reviewed the occurrence of fox rabies in Georgia over a 30-year period and related changes in prevalence of the disease to changes in agricultural practice that influenced the amount of habitat for foxes. He concluded that: "*it seems especially desirable to consider agricultural trends as useful for planning long range programs. If one expects large areas to be abandoned, or altered in their uses, then one might seriously consider the possibility that rabies would increase*".

Urban and, particularly, suburban environments have created a new ecological niche that has been adopted by mesocarnivores, such as red foxes in Europe and Japan, and raccoons and coyotes in North America. Rottcher and Sawchuk (1978) attributed the emergence of the jackal as the dominant factor in the epidemiology of rabies in Zambia to eradication of larger predators and the ability of the jackal to adapt to the food resources available in settled areas. Presence of a high density of mesocarnivores in close proximity to humans has created other zoonotic problems, including rabies, *Baylisascaris* infection, and *Echinococcus multilocularis* infection. Environmental changes that allow adaptable carnivores to thrive are likely to result in increased problems with disease and this should be considered in the evaluation of proposed environmental changes.

It is important to consider the potential side-effects of disease-management programs on other diseases and on the environment. For example, use of

larvicides to kill mosquitoes in wetlands, as might be done as part of the management of West Nile virus or other arboviruses, may reduce the invertebrates that serve as food for wild species such as waterfowl (Pinkney et al. 2000). Immunization for management of one disease has a cost to the animal that might be expressed in various ways such as reduced parental effort (Råberg et al 2000) or even reduced survival (Hannsen et al. 2004). Oral immunization of foxes against rabies provides the best example of the effect of a disease management on another disease as well as for other parts of the ecosystem. The vaccination program has been widespread and successful over large areas of Europe. Coincident with the success of the rabies control program there has been a marked increase in fox populations [the precise contribution of removal of rabies as a major mortality factor, and other factors such as other modifications in human-fox interactions is unclear (Chautan et al. 2000)]. The increased fox population has been associated with an increased infection rate of the tapeworm *Echinococcus multilocularis* in foxes as well as apparent geographic spread of this important zoonosis (Vuitton et al. 2003; Sréter et al. 2003). Increased fox populations also might have serious consequences for prey species, particularly ground-nesting birds.

The aim of an assessment of this type is to identify potential problems in advance and to recommend steps to prevent or mitigate the effect. Overhead transmission lines can be routed away from marsh areas, nutrient rich effluent and sewage can be prevented from entering ponds or the sea, and forests can be managed to minimize the effect of meningeal worm on moose. Each situation demands a careful consideration of all of the potential diseases that might occur and of their known epizootiology. As a closing thought, good biologists do not make major decisions about habitat manipulation without a sound knowledge of the fauna and flora involved and of the probable effects of the changes on them, but such decisions are often made without similar knowledge regarding the microflora and fauna that constitute disease agents.

14.5 Summary

- Disease management through manipulation of the environment requires a more detailed understanding of the ecology of the disease than is required for other forms of management.
- Habitat manipulation may not provide as rapid results as more direct means of dealing with disease agents but the effects are likely to be more long-lasting.
- Nidality is very important in using habitat modification for disease management. The investigator identifies features that restrict the disease to a nidus and the manager may modify these to reduce or prevent the occurrence of disease.

- Environmental manipulation may be used to influence the causative agent, the host population, the population of other species involved in the disease, and inanimate factors involved in disease occurrence.
- Disease control through habitat modification often involves making either-or type decisions, in which the benefit of reduced losses to diseases must be balanced against reduction in some other beneficial factor(s).
- The probable effect of habitat modifications on occurrence and distribution of disease should be part of the environmental assessment of any such action.

15 Disease management through influencing human activities

> "*Public sentiment is everything. With public sentiment nothing can fail; without it, nothing can succeed*". Quote attributed to Abraham Lincoln by (Gilbert 1964)

That wildlife management consists largely of managing people is an axiom among biologists. Almost anything done to control or prevent disease in wild species involves a considerable amount of people management. While involvement of people in disease management has always been important, it will become ever more urgent as the global human population continues to grow, adding about 76 million persons each year to the current level of about 6.4 billion. This growth in human population will necessitate the movement of people into new ecologic regions and uninhabited areas for exploitation of natural resources. There will be expanded cultivation, development of roads, dams and irrigation, all of which will have effects on the occurrence of disease in wild animals and will lead to enhanced transmission of infectious diseases among wild animals, domestic animals and humans. An ever-increasing proportion of humans will live in urban areas. About 30% of humans lived in urban areas in 1950, in 2000 this reached 47%, and by 2030 it is expected that 60% of humans will live in cities (United Nations 2004). This trend is even more advanced in the most developed countries. One consequence is that, increasingly, the human population will be removed from direct contact and understanding of natural processes and much of their understanding will come from sources such as 'nature' television. This will mean that more effort will be needed to explain disease management.

A major problem in trying to manage disease in wild animals through influencing human behavior lies in convincing people that changing their actions is in their own best interest. Short-term gains from ecosystem alteration, such as increased employment and better returns on investment, are much easier to demonstrate than benefits from protecting biodiversity or fresh water. Many human-induced changes in ecosystems, such as increased food production in agricultural systems, improve human health locally at the expense of other systems such as preserving freshwater, and displace the detrimental effects temporally or spatially (Weinstein 2005). For instance, irrigation usually is beneficial locally, at least in the short-term, but may lead

to increased disease transmission (Jardine et al. 2004), soil salinization, accumulation of toxicants in drainwater, and depletion of aquifers, in the longer term. As Weinstein (2005) observed, draining swamps and replacing forests with concrete may be helpful in eliminating human malaria but it is short-sighted if you or someone else runs out of water as a result.

I will not discuss the over-arching problem of human population growth and appetite, nor will I discuss the type of management needed to obtain funding necessary for disease control programs, although that is an essential skill if a program is to succeed; instead I will discuss forms of action more directly related to technical management of disease. I have mentioned at various places earlier in the book that many of the most serious disease problems in wildlife are directly related to some human activity. These usually result from habitat modification or loss, artificial manipulation of animal populations, or because of direct introduction of disease agents or risk factors into the environment. Much of what can be done to control or prevent disease consists of recognizing the potential effects of such activities and trying to prevent or mitigate the effects before they occur, or of trying to reduce or control the effects of some existing activity.

The most simple situations are those in which some man-made element is a direct cause of morbidity or mortality and management consists of removing or neutralizing this factor. For instance, some large birds are particularly prone to collide with overhead wires passing over wetlands, e.g., 38% of mute swans found dead during a long-term study in England died as a result of such collisions (Owen and Cadbury 1975). Care in the location of overhead lines in relation to areas of bird movement and concentration, alterations in the configuration of the wires, and marking of the wires, are modifications that may reduce mortality as a result of collision (Anderson 1978, Meyer 1978). Electrocution on electrical transmission lines also is an important cause of mortality for some birds. Electrocution was the third most common cause of death of bald eagles in the U.S.A. and killed approximately as many eagles as did infectious diseases and intoxication of all types combined (Reichel et al. 1984). Modification of the spacing and arrangement of the wires makes towers carrying such wires more safe as roosting sites by large birds (Miller et al. 1975) and could substantially reduce mortality.

The solution to most anthropogenic problems is less direct. In this chapter, I will deal with three major areas: (i) problems associated with the movement of animals and disease agents, (ii) legislative or regulatory means to reduce or curb disease, and (iii) public education and planning of human activities, to reduce the impact of diseases not manageable in other ways.

15.1 Movement of animals and disease

Humans are compulsive and inveterate movers and transporters of biological materials. We do this consciously to ‘enrich’ the fauna of an area with exotic wild species, such as the house sparrow and the starling, and inadvertently as,

for example, when *Aedes albopictus*, a mosquito vector of many arboviral diseases of man, was introduced to the Western Hemisphere from Asia in used tires (Hawley et al. 1987). In some cases, wild animals may be translocated to promote recovery of populations decimated by disease (Dullum et al. 2005). In transporting biologic materials about the globe we circumvent natural geo-physical barriers that have determined the distribution of animals and disease agents. This process of human-assisted movement has occurred for centuries but the risk of successful translocation of animals and diseases has increased dramatically with developments in transportation. In the past, the rigors of transport were such that many disease agents, vectors, and hosts failed to survive the trip and this, in itself, was a barrier to the spread of disease. However, not all agents are equally susceptible to the rigors of prolonged travel and some diseases such as plague were transported widely about the world in sailing ships and in the baggage of armies and caravans. The situation is now much more conducive to effective translocation of disease, with travel time between any two points on earth, even by commercial airlines, being within the lifespan of most arthropod vectors and shorter than the incubation period of most infectious diseases. This time period often is so short that even fragile agents may survive in the relatively inhospitable environment of soil clinging to boots or equipment. Much of what I will be discussing in this section deals with creation of artificial barriers to replace the natural barriers to disease movement that can now be circumvented so easily.

The movement of agents and/or animals may create at least three types of disease problem. The first problem, and the most obvious, occurs when a disease agent is introduced into an area where it did not previously occur. If the disease agent becomes established at the new site it may have a serious impact on indigenous species, including humans and domestic animals. A second, less obvious problem occurs when highly susceptible animals are introduced into an area where a disease agent, to which they are vulnerable, already exists in the native fauna. The indigenous disease may have a serious impact on the introduced species, although it may cause little or no detectable problem in native animals. The third potential problem occurs when the introduction of a new species changes the ecology of an existing disease or host–parasite relationship, so that it becomes more of an issue. The first of these three potential problems is by far the most serious but the second may result in the failure of costly transplantation exercises. In both the second and third situations, the introduction of new animals may result in management becoming necessary for a disease that was previously of no particular significance.

15.1.1 Introduction of novel disease agents

Many examples are available of diseases that have become established in new areas as a result of translocation of wild animals (Table 15.1) and it is worthwhile considering a few of these in detail.

Table 15.1 Examples of diseases that have been moved through the translocation of wild animals

Disease or agent	Type of movement
Bovine tuberculosis	Alberta to Wood Buffalo National Park with bison.[1]
Oedemagena tarandi	Norway to Greenland with reindeer[2]
Malignant catarrhal fever	Africa to North America with wildebeest[3]
Raccoon rabies	Florida, Georgia to Virginia with raccoons[4]
Elaphostrongylus cervi	Europe to New Zealand with red deer[5]
	New Zealand to Australia with elk[6]
Echinococcus multilocularis	Northern to southeastern USA with foxes[7]
Dog rabies	Texas to Florida with coyotes[8]
Exotic ticks	Tropical areas to North America with reptiles[9]
Plague	China to western North America with rats[10]
Elaphostrongylus rangiferi	Norway to Newfoundland with reindeer[11]
Fascioloides magna	North America to Europe with elk[12]
Squirrel parapoxvirus	North America to Britain with grey squirrels[13]
Aleutian mink disease	North America to Europe with mink[14]
Avian influenza (H5N1)	Thailand to Belgium with smuggled eagles[15]

[1]Fuller (2002), [2]Clausen et al. (1980), [3]Castro et al. (1982), [4]Baer (1985), [5]Watson and Gill (1985), [6]Presidente (1986), [7]Davidson et al. (1992), [8]Anonymous (1995), [9]Burridge et al. (2000), [10]Gaspar and Watson (2001), [11]Lankester (2001), [12]Pybus (2001), [13]Tompkins et al. (2003), [14]Fournier-Chambrillon et al. (2004), [15]van Borm et al. (2005)

Nematodes of the genus *Elaphostrongylus* have a wide distribution in cervids in Eurasia (Steen and Rehbinder 1986; Lankester 2001) and utilize a variety of gastropods as intermediate host. The adult worms may invade the nervous system of cervids and cause severe neurologic disturbance, including paralysis and blindness. Neurologic disease caused by *Elaphostrongylus* occurs in red deer (Borg 1979; Watson 1983), reindeer (Kummeneje 1974), caribou (Lankester and Northcott 1979), sika and maral deer (Watson and Gill 1985) and moose (Steen and Rehbinder 1986; Lankester 1977). In addition

to the propensity to cause neurologic disease, the parasite may also cause interstitial pneumonia (Sutherland 1976). Carcasses of farmed red deer have been condemned because of lesions in the intermuscular fascia caused by *E. cervi* (Mason et al. 1976) and the carcass weight of adult moose infected with *E. alces* is significantly lower than that of uninfected animals (Stuve 1986). This is not the type of parasite that one would knowingly transplant into new areas where susceptible cervid species are present; however, there is a growing history of that having been done.

Elaphostrongylus cervi was recognized to be present in New Zealand in 1975, when infection was found in red deer (Mason et al. 1976) and elk (Mason and McAllum 1976); neither of which is native to New Zealand. Watson and Gill (1985) suggested that the parasite could have been introduced to New Zealand either with elk from North America or with red deer from Scotland. The latter source is far more likely, as *E. cervi* is enzootic in red deer in Scotland but has not been found in elk in North America. Introduction probably occurred at about the turn of the 20th century, before the parasite had been discovered.

In 1986, *E. cervi* infection was diagnosed in one of a group of 33 elk being held in quarantine in Australia after importation from New Zealand. The infected animal was destroyed and further importation of live deer from New Zealand was suspended (Presidente 1986). This case is notable for two reasons. The first is that it represents one of the few documented examples in which an exotic disease agent was recognized during the transplantation process and dealt with before release of the animals. It also is important because of the extent of the measures that had been taken to ensure that the animals were not infected with the parasite prior to importation. All 170 deer on the farm of origin in New Zealand had been examined and were negative for larvae of *E. cervi* when tested prior to selection of animals for export to Australia. The animals selected for export were then quarantined on pasture for 6 months, separate from other deer, then treated each day for 5 days with an anthelmintic and, finally, held in quarantine off pasture for a further 42 days prior to export. After arrival in Australia, the elk were placed in quarantine for 100 days and feces were examined from each animal on three occasions (4, 40, and 69 days after arrival). Larvae were detected only on the third examination.

The second occurrence involved 1,597 red deer in four groups imported into Canada from New Zealand (Gajadhar et al. 1994). Feces were collected from each animal within 30 days of arrival in quarantine. A total of six animals in three of the herds were found to be infected with *E. cervi*. When repeated fecal samples were taken from these animals, larvae could not be detected consistently. All four herds were depopulated and importation of cervids from countries where *E. cervi* is known or suspected to occur was suspended (all of the red deer had been negative on one to three tests done while in quarantine prior to leaving New Zealand).

Many factors, including a long prepatent period [up to 206 days, Gajadhar et al. (1994)], intermittent shedding of larvae, and suppression of larval output

by anthelmintic treatment, may have been involved in these cases, but they serve to illustrate several points. The first is the extreme difficulty in detecting and preventing entry of certain diseases when live animals are translocated. **A living animal cannot be readily separated from its microflora and microfauna for purposes of translocation and it is impossible to sterilize a living animal**. The measures required by the Australian government, in particular, were very rigorous and, no doubt, were considered excessive by those interested in moving the animals. But the measures were not sufficiently rigorous to prevent movement of the parasite. The second point worth noting is that drug treatment was ineffective in ridding the Australian animals of the infection, although it may have stopped larval output temporarily and, hence, made the parasite even more difficult to detect. Chemotherapeutic agents are seldom 100% effective, even under ideal conditions. To complicate the matter further, very few drugs have been tested specifically in wild species. Fortunately in both cases described above the parasite was recognized prior to its release. This demonstrates the value of an extended quarantine period, with careful monitoring prior to release. It must be noted that the measures used to prevent introduction of *E. cervi* into Australia were markedly more stringent than those required in most instances in which wild animals are transplanted into new areas.

An incident involving another parasite in the same genus is worth reviewing. *Elaphostrongylus rangiferi* was detected in wild caribou on Newfoundland, Canada in 1976 (Lankester and Northcott 1979, Lankester 2001). It is thought to have been brought with reindeer from Norway to the island in the early years of the 20th century. Cases of neurological disease have been found in naturally infected caribou in Newfoundland, and a moose infected experimentally with parasites derived from caribou developed neurologic disease (Lankester1977). *Elaphostrongylus rangiferi* is not known to occur elsewhere in North America, although caribou from Newfoundland were transplanted to Maine in the 1960s and in 1987. The early introduction failed and the animals died. The animals moved in 1987 were treated with an anthelmintic prior to translocation but larvae, that may have been those of *E. cervi*, were shed by two animals after arrival in Maine. The animals were then treated rigorously with anthelmintic and held in quarantine (M.W. Lankester, personal communication). The ultimate fate of the animals and worms is unclear. It must be noted that transplantation of caribou to Maine in 1987 occurred despite knowledge of the presence of the parasite in Newfoundland caribou and after the results of the Australian experience with *E. cervi* had been published, so that the indifference of those involved in the transplantation cannot be excused.

There have been multiple translocations of rabies virus with wild animals. The best known of these was introduction of rabies with wild-caught raccoons purchased from animal dealers in the southeastern USA that were transported and released by hunting clubs in more northern areas. The success of the release programs was extremely poor. In one survey, only 3.1% of the released animals

were recovered by hunters, at a cost of $640/animal. Most animals died shortly after release (from a disease-management perspective, failure of the transplants was likely a desirable result, but the unnecessary death of the animals cannot be condoned). Many disease agents were documented in the translocated raccoons, including protozoa (Schaffer et al. 1978), helminths (Schaffer et al. 1981), parvovirus (Nettles et al. 1980) and rabies virus (Nettles et al. 1979). Many of these disease agents were not present in indigenous raccoons at the proposed release sites. The introduced rabies virus resulted in a major epizootic involving many states and southern Canada. Two other examples of translocated rabies are the establishment of rabies on several Caribbean islands with introduced Indian mongooses and movement of dog-strain rabies in coyotes moved from Texas to Alabama and Florida for hunting preserves (Rupprecht et al. 2001).

The experience with *E. cervi*, described earlier, demonstrates the value of a strict quarantine period, during which the animals are monitored closely, after they reach their destination but before they are released. The value of such a quarantine period was also evident in New Zealand where the winter tick *Dermacentor albipictus* was detected on two occasions on elk imported from Canada, while they were being held in quarantine (Heath 1986). **For quarantine to be effective, animals must be held for at least as long as the maximum known incubation period for any of the diseases that they might be carrying**. Thus, if the maximum recorded prepatent period (the period from infection until eggs or larvae are passed) of a parasite is 100 days, animals suspected to carry the parasite must be held in quarantine, and examined regularly, for at least 100 days. Calvete et al. (2005) made a number of suggestions for improving quarantine, from the perspective of increasing the survival among quarantined animals.

It is appropriate to prohibit or prevent the translocation of any wild animal until the risks of disease transfer have been assessed fully. Corn and Nettles (2001), Leighton (2002), and Armstrong et al. (2003) provide detailed information on doing a risk assessment. If translocation is still considered to be desirable after such an assessment, it should proceed only when suitable diagnostic and quarantine measures are available and can be applied. This is not an area where policy can be flexible if the aim is to prevent disease introduction. Rigid application of this basic principle has been the backbone of control measures to prevent the international spread of livestock diseases and it has proven to be remarkably efficient for that purpose.

15.1.2 Introduction of animals susceptible to indigenous disease agents

Serious disease may occur among exotic animals introduced into an area where a disease agent is indigenous. Examples in which this has happened are shown in Table 15.2. *Parelaphostrongylus tenuis*, a nematode closely related to *E. cervi*, will be discussed as an example. The normal host of *P. tenuis* is the

Table 15.2 Examples in which an indigenous disease in wild animals at the release site has had a negative effect on translocated wild animals

Disease or agent	Introduced species	Source of infection
Aspergillosis	Penguins	Temperate zone birds[1]
Avian malaria	Penguins	Temperate zone birds[2]
Parelaphostrongylus tenuis	Reindeer/caribou	White-tailed deer [3]
	Black-tailed deer	White-tailed deer [4]
Elaphostrongylus cervi	White-tailed deer	Red deer [5]
Schistosomiasis	Atlantic brant	Indigenous waterfowl[6]
Eastern equine encephalitis	Ring-necked pheasant	Indigenous birds[7]
	Whooping crane	Indigenous birds [8]
	African penguin	Indigenous birds [9]

[1]Kageruka (1967), [2] Griner and Sheridan (1967), [3]Anderson and Prestwood (1981), [4]Nettles et al. (1977), [5]Kotrly and Ehrardova- Kotrla (1971), [6]Wojcinski et al. (1987), [7]Beaudette et al. (1952), [8]Dein et al. (1986), [9]Tuttle et al. (2005)

white-tailed deer, in which the worm causes little or no clinical disease. However, the parasite produces severe and often fatal neurologic disease in a variety of other cervids, as well as in some domestic ruminants. Some attempts to establish populations of other cervids, notably caribou and reindeer, in areas where the parasite is enzootic have failed because the introduced animals died of neurologic disease caused by the worm (Anderson and Prestwood 1981). The llama also is very susceptible to the parasite and parasite-induced neurologic disease is common in llamas in areas where the parasite occurs in deer (Baumgartner et al. 1985).

Changes in habitat in eastern North America that occurred with settlement allowed expansion of the range of both the white-tailed deer and of the parasite with serious consequences for other cervids in these areas (Anderson and Prestwood 1981). Caribou were particularly affected. In this situation, the parasite moved and affected indigenous animals. This parasite must be considered seriously in any program involving the transplantation of cervids in North America, because of: (i) the risk of transplanting the parasite to areas where it currently does not occur, and (ii) its probable effect on exotic animals introduced into areas where the parasite is enzootic. *Parelaphostrongylus tenuis* is enzootic in white-tailed deer in Maine, so that the transplantation of caribou from Newfoundland, described earlier, is a good example of doubly bad practice. The introduced caribou were likely to succumb to *P. tenuis*, perhaps before the exotic nematode, *E. rangiferi*, which might be introduced with the caribou, became a problem for native cervids.

Occurrence of an indigenous disease affecting introduced animals hampered efforts to propagate whooping cranes in captivity. The virus of eastern equine encephalitis is transmitted by mosquitoes and is enzootic in many

areas of eastern North America, where it produces transient, sub-clinical, infection in native birds. However, the virus produces fatal disease in a variety of introduced species, most notably whooping cranes and ring-necked pheasants. An outbreak of encephalitis in 1984 killed whooping cranes in a captive propagation program at the Patuxent Wildlife Research Center in Maryland. The discovery that the disease is enzootic in the area was considered a serious risk to the propagation program for this endangered species and led to development of a vaccine (Clark et al. 1987). It will never be possible to predict all such effects in advance, but the presence of diseases in native fauna of the recipient area always should be considered in any plan to translocate wild animals.

15.1.3 Introduction of a species that alters the ecology of an indigenous disease

This aspect of translocation has received relatively little attention but addition of a new species that acts as a host for an existing disease agent can have major effects on disease. The most dramatic example of this has been the introduction of brushtail possums to New Zealand and the subsequent impact on bovine tuberculosis. Bovine tuberculosis has been controlled effectively or eliminated in many parts of the world by measures directed at domestic cattle; primarily through test and slaughter. However, tuberculosis has proven impossible to control in some countries, because of the existence of an alternate wild host for the disease. Tuberculosis occurred in cattle in New Zealand prior to the introduction of the brushtail possum from Australia as a potential fur-bearing animal. Possums became a serious environmental pest, because of damage to native forests and because it became the primary reservoir for *M. bovis*. The population of possums is estimated to be about 70 million animals. Control measures that have been effective in other parts of the world for eliminating tuberculosis from cattle have failed in New Zealand and continuing transmission of *M. bovis* from possums to cattle is the single greatest barrier to eliminating the disease in domestic livestock (O'Neil and Pharo 1995).

A less dramatic example of the impact of an introduced species occurred on the arctic archipelago of Svalbard. Although arctic foxes were present, including winter migrants infected by the adult stage of the tapeworm *Echinococcus multilocularis*, this zoonotic agent did not become established, because of lack of a suitable rodent intermediate host. However, when sibling voles were introduced, perhaps in forage for livestock, all of the required elements were present and the parasite became established. "*This is an interesting example of how an accidental introduction of an intermediate host can contribute to the establishment of a dangerous parasite*" (Hentonnen et al. 2001).

Introduced animals may alter an established disease in other ways. Introduction of a less competent host species may reduce the prevalence of

certain diseases in the primary host through a "*dilution effect*" (Ostfeld and Keesing 2000). This only pertains to diseases that require an intermediate host. An example that appears to fit this hypothesis has been reported by Telfer et al. (2005). Native wood mice in Ireland are infected with two species of *Bartonella* that are transmitted by fleas. The introduced bank vole is infested by the same flea species as the wood mouse but has not been found to be infected with *Bartonella* in Ireland. In areas where bank voles have become established, the prevalence of *Bartonella* in wood mice is lower than in areas without bank voles, and the prevalence in wood mice is inversely proportional to the density of bank voles.

15.1.4 General comments about translocation

It is impossible to move or transfer live animals without also transferring potential disease agents. Fortunately, many introduced diseases fail to become established in the new environment and others, that have become established, may be of little recognizable consequence at this time. However, good fortune is no substitute for good management and one should always be conscious that imported diseases have had disastrous consequences in the past. Probably the most dramatic documented example was the introduction of rinderpest into Africa with Zebu cattle from India. This resulted in a devastating epizootic among wild ungulates that swept the length of the African continent. "*It was estimated, for example, that 90 percent of the buffalo in Kenya died, and that the bongo were almost exterminated*" (Henderson 1982). An important indirect result of rinderpest introduction was that the reduction in another disease, trypanosomiasis, that occurred in association with the absence of game animals lead to a policy of systematic "*game destruction*" in southern Africa for the control of that disease in livestock (Henderson 1982).

It is nearly impossible to totally prevent inadvertent introduction of vectors or agents that may travel as passengers in old tires, on peoples' shoes, or in or on other fomites, except by the stringent type of controls now in effect to prevent introduction of human and domestic animal diseases. Sanitation measures, such as inspection of the belongings of immigrants and control of garbage from ships and aircraft, also protect wild animals, although that is not their intended purpose. It is possible; however, to reduce or prevent the introduction of new diseases that may travel with transplanted wild animals. The simplest, and the best way to prevent introduction of diseases is to disallow importation of live animals from any area where an exotic disease might, or is known to, occur. However, this requires knowledge of the occurrence of specific diseases in individual species and of the geographic distribution of diseases. This information often is not available for wild species.

If no information is available on the occurrence of disease in the donor population, there are a number of choices. The first and most obvious option

under such circumstances is to decide that the risk of introducing a disease outweighs the potential benefits and forego transplantation. Another option would be to import only reproductive products (fertilized ova and/or semen) rather than live animals. This method circumvents many of the problems associated with certain types of disease and has been used extensively for transfer of genetic material from domestic livestock, as well as from deer for game farming. However, it is likely not appropriate for many situations in wild animals. A third option would be to screen the donor population carefully to ensure that the individual animals chosen for translocation are free of specific diseases. However, as discussed earlier, **one can never be certain that a population is free of disease without testing every individual using a test that is 100% reliable**. In most circumstances, the only available option, where translocation is considered to be necessary and unavoidable, is to sample each individual animal that is a candidate for translocation. For this to be effective, a reliable and highly sensitive method for detecting infected individuals must be available. This often is not the case in wild animals and the efficacy of most screening techniques is unknown. For some diseases, such as rabies, there is no suitable method for testing live animals.

In many cases it is logistically impossible to test all of the individual animals that might be moved and the best that can be done is to examine a sample. It is critical that the disease specialist explain, in advance, that **negative results on a sample do not guarantee freedom of disease**. All that can be reported is the maximum prevalence of disease that can be detected using the sample examined. For example, assume that 350 wild birds are to be translocated but it is only possible to obtain samples from 30 birds (8.6%). If none of these 30 birds tests positive, the minimum prevalence of disease that could be detected at the 95% confidence interval with this sample size is 10%. Stated in another way, the disease specialist could report that based on the sample it is possible to be 95% confident that the prevalence in the entire group is not greater than 10% (see Chap. 8 for discussion of the methodology). In addition, this prevalence is the apparent prevalence rather than the true prevalence, unless the specificity and sensitivity of the tests are known for the species. Thus, the test is not an assurance of absence of disease but it can be helpful in estimating the degree of risk inherent in the translocation.

In addition to problems in detecting known diseases, one always must be cognizant that disease agents that are currently unrecognized also may be translocated. "*It is relatively easy to legislate for known disease, especially where there is a thorough knowledge of its epizootiology, but impossible to do so for unknown disease or those where knowledge of the epizootiology is lacking*" (Biggs 1985).

No movement of animals should proceed until these questions have been answered and suitable methods have been established to prevent the occurrence of disease as a result of the translocation. Most biologists would not proceed with translocation of animals without understanding how the animals would affect and be affected by the flora and fauna at the release site.

However, translocations are often made without consideration of the microfloral and microfaunal organisms that may cause disease.

15.2 Modifying human activities by regulation and legislation

Any attempt to control disease in wild animals inevitably involves people in some way. In much of the world, most wild animals belong to the people and are managed in the public interest, so that an agency must have support from the public to succeed in any management program. People must know and understand what is being done, how it will be done, and why it is being done, before they will support the action. There are two basic ways of modifying human activities and behavior, either through some form of compulsion, such as legislation or regulation, or by education and persuasion. Although the two methods may seem distinct, it is important to remember that regulations are created by elected officials who respond to public opinion and who need to be educated about the need for regulations, and that unpopular regulations will be ignored or flaunted. Thus, it is important that the public is informed and supportive of the action. A large segment of the general public is interested in wild animals and "*many of these people will react if they think wildlife is being mistreated or if they think some agency is planning to do something detrimental to the resource*" (Shay 1980). Public opinion can be very much of a double-edged sword in regard to management of wildlife diseases. Public support has been used effectively to promote legislation and regulations to reduce or control a number of serious disease problems caused by environmental pollutants, such as mercury and certain pesticides. However, there may be marked negative public reaction to management that requires population control or severe habitat manipulation through techniques such as prescribed burning or clearing. Such instances require extensive advance education of the public so that they understand how and why the action will be taken.

The severe controversy that erupted when an 'emergency' population reduction of deer was attempted in Florida serves as a case-study of problems that can occur when there is no time for such education. Torrential rains during the summer of 1982 confined the large deer herd to small islands of habitat in the Everglades. "*Based on projected water levels and past experience with the deer herd under similar circumstances, Commission biologists predicted extensive deer mortality unless the herd could be quickly reduced to a level commensurate with the habitat conditions*" (Florida Game and Fresh Water Fish Commission 1983). Emergency hunts were authorized to reduce the herd from 5,550 to 2,300, a level considered appropriate for the resources available. There was immediate opposition from groups who wished to stop the hunt and use capture/relocation or feeding of deer as alternative remedies, although these had proven unsuccessful during similar circumstances in

previous years. After extensive legal delays, opponents were allowed to attempt rescue of deer on a portion of the area while a hunt proceeded in the remainder of the area. During hearings, potential rescuers had testified that 2,000 deer could be removed in 8 days but the rescue was halted after 1 ½ days when only 18 deer had been captured. The rescuers admitted that it was impossible to remove enough deer to have an impact on the population. About 67% of deer present in the area where rescue was attempted died, while about 23% of deer in the hunted area died of natural causes or were killed by hunters. It was concluded that: "*wildlife management practice, no matter how well-founded on biology and management principles, can become highly controversial if it is not understood and accepted by the media and the general public*" (Florida Game and Freshwater Fish Commission 1983).

Public acceptance and approval usually is high for short-term remedial actions, such as feeding starving deer or rehabilitation of injured or oiled birds. However, it can be argued that this type of emergency disease response may be deleterious to sound management, because it diverts attention and funding away from the more basic factors, such as too many deer in too little habitat, that caused the problem. Promoting actions such as emergency feeding also may create a perception that the problem is under control and that the short-term emergency response is the appropriate way to deal with the situation. Some types of emergency treatment provide an opportunity to educate the public about the cause and nature of disease. This can be used to make the public more receptive to management designed to prevent disease recurrence. For example, sportsmen who participated in an emergency winter feeding program for deer in Saskatchewan became aware that the root cause was insufficient winter habitat. They then became strong proponents for habitat improvement. Similarly, while the number of birds saved and returned to the wild during a cleanup operation after an oil spill may be insignificant biologically, the publicity and surveillance that results may have some deterrent effect on potential polluters. Public concern generated by the exercise also may be a powerful tool to convince legislators of the need for more stringent preventive regulations.

Legislation has been particularly effective for the control of environmental toxicants and for preventing importation of exotic diseases with introduced domestic animals, as was discussed earlier. Very few regulations have been drafted specifically to prevent introduction of diseases of wildlife. A few examples are available of regulations related directly to wildlife diseases. These include a longstanding policy of not allowing importation of hares into Denmark to prevent introduction of tularemia (Bendtsen et al. 1956), decisions not to introduce and release Arabian oryx with bluetongue antibodies into Oman, or to release captive orangutans exposed to human tuberculosis in Indonesia (Jones 1982). The continuing movement of wild animals, often for trivial purposes, emphasizes the need for more such regulations.

Legislation may also be used to reduce the risk of exposure of the public to certain zoonotic diseases of wild animals. A law was introduced in Oklahoma

in 1977 that made it illegal to remove the scent glands or to vaccinate skunks for the purpose of domestication, after three pet skunks exposed 42 people to rabies. Legislation was enacted in 1995 prohibiting movement of foxes, coyotes, and raccoons within or out of Texas as part of a program to control rabies (Sidwa et al. 2005).

15.3 Modifying human activities through education

Legislation must be accompanied by appropriate public education to ensure that the reason for the control is understood and that the regulations will be obeyed. Almost any procedure to manage disease in wild animals will benefit if the public understands why and how the program will be done. In many instances public education and acceptance may be critical to the success of a program and, in other situations, modification of human activity may be the most efficient method for managing a disease. Management of hydatid disease (infection with the larval form of the tapeworm *Echinococcus granulosus*) in various parts of the world provides an example of the value of education in a disease control program. This parasite has a two host life-cycle, with the adult tapeworm occurring in the intestine of a carnivore and the larval form (hydatid) in tissues of a herbivore. Humans may become infected with the larval stage, which forms large cysts in tissue, including in lung, brain and liver. The disease is a serious zoonosis in many parts of the world. In most areas, the parasite cycles between domestic livestock, particularly sheep, and dogs. Control consists largely of measures to prevent dogs from gaining access to infected sheep tissues and hygiene to block transmission from dog to man. During the 19th century, the disease was enzootic in sheep and dogs in Iceland and approximately one-sixth of the human population was infected (Schantz and Schwabe 1969). The first measure taken for control of the disease was distribution of a pamphlet describing the nature, cause and means of prevention of the parasite to every family in 1864. The same information was taught at all levels in schools, so that every individual in the country became fully familiar with the disease. This resulted in voluntary control measures that were so effective that the prevalence of infection in humans fell from 15–22% to 3% by 1890, when compulsory control was introduced. The parasite was eradicated from Iceland by the early 1950s.

In contrast, early attempts to control hydatid disease in New Zealand involved only sporadic educational efforts and were based on legislation that made it illegal to feed raw sheep tissue to dogs. This was regulation with only limited explanation of the reasons for the regulation. These measures had little effect on the prevalence of the disease, probably as a result of failure of people to comply with the regulations. A more intensive educational campaign, that involved teaching about the parasite in schools and stressing zoonotic aspects to farm wives led to voluntary farmer-initiated hydatid

eradication campaigns. These were coordinated by the government and resulted in a very marked reduction in prevalence of the disease. New Zealand was able to declare provisional freedom from hydatid disease after a campaign the lasted >50 years (Pharo 2002).

In reviewing the management of hydatid disease, Schantz and Schwabe (1969) stated that "*control is largely a question of people's determination. In effect, the local population must be educated to the dangers of the disease and motivated to do something about it*". This statement could be applied to almost any disease-management program. It is important to remember that technical knowledge is usually far ahead of public knowledge and acceptance.

Once a disease-management program has been accepted and begun, continual feedback of information is necessary to maintain enthusiasm for the program. This feedback must be to those involved directly in the program, to the politicians responsible for funding the work, and to the public. Information on progress of the project becomes particularly important in the latter stages of a successful campaign, when the disease has nearly been controlled and it is no longer highly visible. Under these circumstances, the management program may no longer appear to be a priority for funding, and general enthusiasm and effort may wane, allowing recrudescence of a partially vanquished disease. This appears to have occurred during a program to control rabies in skunks in Alberta. A population reduction campaign had reduced the population density of skunks and the prevalence of rabies in a focal area but had not eradicated the disease. Attention was then shifted to a new problem area, with a concomitant decrease in effort in the original focus. Both the skunk population and the prevalence of disease rebounded in the original area.

Public education can be used in other ways to reduce the **effect** of a disease, without having to control or reduce its prevalence in wild species. This is particularly appropriate for many zoonotic diseases, such as rabies, trichinosis, giardiasis, plague, and some arthropod-borne viral infections that are enzootic in wild animals. These disease agents present little or no problem when confined to animals but become a problem when people are exposed. Education in such situations is usually directed toward acquainting people with the occurrence and nature of the disease in wild animals, and to suggesting methods for avoiding potentially hazardous situations and preventing disease transmission. In the case of rabies, such an educational program might include advising the general public to: (i) avoid wild animals that seem tame, friendly or that are otherwise acting abnormally, (ii) report animals of this type to appropriate authorities, (iii) consult a physician immediately if they think they have been exposed to such an animal, (iv) encourage regular vaccination of domestic pets, and (v) discourage keeping of wild animals, particularly skunks and raccoons, as pets. In the case of arthropod-borne diseases, education is usually directed at methods to reduce exposure by encouraging use of insect repellents, the wearing of appropriate clothing, protecting susceptible infants from insects, and treatment of pets to prevent them introducing rodent fleas and ticks into the home.

Special programs may be needed to reach and educate particular groups within the population that are at greater risk than the general public. For example, a special education program might be used to advise bear hunters of the occurrence of *Trichinella spiralis* in bear meat and of appropriate methods for caring for the meat. An information package of this type could be distributed directly with the license for hunting bears. Trappers might be advised of special precautions, such as not skinning animals found dead and wearing rubber gloves while handling animals, to reduce the risk of contracting tularemia and rabies. Conservation officers and pest control officials, who may have to deal with rabid animals, should receive intensive instruction on how to avoid exposure to the disease and should be vaccinated prophylactically. Campers and hikers using areas where zoonotic diseases, such as plague and giardiasis, occur might be provided with specific information to reduce the likelihood of exposure. The objective of such education is to reduce the risk of exposure without creating hysteria or aversion to outdoor activities.

The same basic educational methods can be used to reduce transmission of disease from wild to domestic animals. For example, a program to educate farmers about the risk of feeding hare viscera (potentially infected with *Brucella suis* biotype 2) to swine, was proposed as the best method of controlling porcine brucellosis in Denmark (Christiansen and Thomsen 1956). Promotion of isolation of domestic poultry from contact with wild birds has been recommended for many years as part of the program for control of influenza in poultry (Wood et al. 1985) and continues as part of the current programs to deal with H5N1 influenza. In these instances, the education campaign is intended to reduce the risk of transmission to livestock without having to reduce the prevalence of disease in wild species.

15.4 Integrating disease management in planning

An important aspect of disease management is the use of features of the ecology of both the disease agent and the wild animals in planning human activities, in order to reduce disease risk. The features that often are of greatest value in this regard are the spatial and temporal distribution of the agent and of the hosts. Many diseases are distinctly seasonal and have a high degree of nidality. As an example, avian botulism is strongly associated with hot weather, so that management procedures, such as alterations of water level, which might provoke an outbreak, should be avoided in the high-risk summer period. One would not recommend a rough-fish poisoning program on a botulism-prone marsh during warm weather, as the dead fish could provide abundant substrate for growth and toxin production by *C. botulinum*. Similarly, public use of a campground might be scheduled to avoid the seasonal occurrence of large numbers of ticks in areas where tick-transmitted zoonoses, such as Lyme disease, are a problem.

Disease-management activities, such as closure of campgrounds or public information campaigns, can be timed more precisely to coincide with periods of high risk if the occurrence of the disease is monitored in wild or sentinel animals. The latter technique, employing groups of sentinel chickens, has been used for many years to provide early warning of an increase in the amount of certain arthropod-borne viral diseases present in an area (e.g., Nichols and Bigler 1967; Morgante et al. 1969). When increased viral activity is detected by increased infection rate among sentinel birds, appropriate preventive measures, such as a public advisory, can be put in place. Similarly, Valtonen et al. (1980) suggested that the probability of epizootics of tularemia in Finland could be predicted by monitoring fluctuations in the number of wild rodents.

Knowledge of the biology of wild species that may be affected also can be used in planning human activities that might result in disease among wild animals. For example, Yom-Tov (1980) proposed a method of timing bird control operations in irrigated fields that was both efficacious in terms of reducing pest species, and had a minimal probability of causing secondary poisoning of raptors. The simple principles outlined by Yom-Tov should be applicable to many other uses of pesticides in agriculture. Unfortunately, most applications of pesticide are timed with the pest in mind and with little or no consideration of the phenology of events in other species that may be affected adversely.

If a zoonotic disease has a known nidality, it may be possible to direct human activities away from such sites. McLean et al. (1981) characterized the landscape epidemiology of Colorado tick fever (CTF) and found that one nidus containing "*the most rodents, ticks, and CTF virus*" was located within a large public campground. They concluded that there was "*a high risk of CTF exposure to campers, especially in May and June*". This information could be used to direct campers away from such areas during periods of high risk and also in choosing sites for future campgrounds. Human activities often unintentionally create a nidus for disease. Human artifacts, such as rock walls, refuse heaps, and streamside rip-rap, created habitat for rock squirrels in towns of the southwestern U.S.A. This increased both the population density of squirrels and the risk of human plague (Barnes 1978). In this case, a program to reduce the risk of plague might require an extensive public education program to explain the relationship among habitat, squirrel density, and the disease. The squirrels were considered an attractive part of the fauna and it was thought that any direct attempt to reduce their numbers would be resisted (Barnes 1978).

15.5 Disease transmitted from humans to wildlife

A few diseases are transmitted directly from humans to wild animals in nature. Examples of this type of situation include *Mycobacterium tuberculosis* infection in suricates and banded mongoose in South Africa, likely as a

result of exposure to infectious human sputum (Alexander et al. 2002) and cryptosporidiosis in non-human primates in Sri Lanka (Ekanayake et al. 2006). In these situations, the occurrence of the disease in the wild animals usually is a minor problem compared to occurrence of the disease in humans, and it is probable that management directed to reduce infection in humans also will be beneficial for wild animals. Occasionally it may be possible to control or prevent disease transfer directly. Ferrer and Hiraldo (1995) found that the occurrence of *Staphylococcus aureus* infection in nestling eagles could be reduced greatly by requiring that handlers banding the birds wear disposable gloves.

15.6 Summary

- Management of disease in wild animals usually involves a high degree of people management.
- The public must understand the reason for the management and how it will be done if they are to support it. Public education should be a part of any major program.
- Management programs should include continual feed-back to all involved, and to the public, to maintain enthusiasm for the project. This is particularly important in the later stages of successful campaigns, when disease management may cease to be a priority and the disease may be allowed to re-emerge.
- Translocation of wild animals is a management activity that involves a high degree of risk from disease.
- Translocated animals may introduce exotic diseases that will adversely affect indigenous species, or translocated animals may be affected adversely by disease agents present in indigenous animals.
- Introduced animals may alter the ecology of an existing disease.
- Animals should not be translocated without an understanding of the potential disease agents present at both the site of origin and the release site. Every translocation should be subject to a formal risk assessment.
- It is impossible to separate living animals from their microflora and microfauna.
- Treatment with drugs prior to translocation reduces but does not eliminate the risk of transferring disease agents.
- Animals that are translocated should always be held in strict quarantine with regular monitoring after they have been moved but before they are released.
- Knowledge of the spatial and temporal features of wild populations and their diseases can be used in planning human activities to reduce risks to people from zoonoses, and risks to the animals from activities such as pesticide application and human infections.

16 Emergency and integrated management programs

"*It is not a question of whether or not FMD* [foot-and-mouth disease] *or other exotic diseases will gain entry into countries now free of these diseases; but when this will occur*"

(Sutmoller 1984)

16.1 Emergency disease control

Most of the methods discussed in earlier sections have dealt with the long-term management of a disease or with its elimination over time. However, there are situations in which it is necessary to embark on an immediate, emergency management program. The most dramatic examples of this occur when an exotic disease that affects either domestic livestock or humans, such as foot-and-mouth disease or highly pathogenic avian influenza, is identified in an area previously free of the disease. The threat of this type of disease introduction is always present. Most exotic diseases of livestock also infect wild animals, so that wild species must be considered in any emergency management program for livestock diseases. Similarly, many important zoonoses involve wild animals. The general principles developed for the control of livestock diseases would apply to the emergency management of an exotic disease restricted to wild animals, if such control were considered necessary.

The preferred method for dealing with introduced foreign diseases of livestock is containment, followed by eradication as rapidly as is possible. This often is referred to as 'stamping out' the disease and is usually done through restricting movement of animals and animal products, slaughter and disposal of infected and exposed animals, and disinfection of contaminated premises, within an area surrounding the site where the disease was first recognized. This technique has been highly successful in dealing with outbreaks of exotic disease in many countries and, although it requires a massive effort and is tremendously expensive, benefit:cost analyses indicate it to be the method of choice for dealing with exotic diseases, such as foot-and-mouth disease (James and Ellis 1978; Power and Harris 1978; Krystynak 1985). It is probable that the same general method would be used if wild animals were involved in such an outbreak; however, methods for dealing with wild species are less

clearly defined than for domestic animals and most such methods have not been refined through actual use and experience.

The success of any emergency control program is dependent upon the ability to act swiftly and effectively, as the number of animals and the geographic area involved are likely to increase with each day of delay. Two features essential to the success of such programs are: (i) the early detection of the disease, and (ii) the presence of a well organized infrastructure, so that the necessary resources from a variety of agencies can be mobilized, coordinated and deployed quickly.

The initial recognition and reporting of diseased animals is "*probably the weakest link in our ability to deal with an outbreak of exotic disease*" in domestic livestock (Sutmoller 1984). For instance, in the 2001 outbreak of foot-and-mouth disease in Great Britain the "*epidemic was already well established and disseminated*" with at least 29 farms infected but undiagnosed at the date of initial confirmation (Morris et al. 2001). This is an even greater problem when dealing with disease in wild animals. Domestic species are observed regularly by their owners and, in the developed world, veterinary care and diagnostic laboratory services are readily available. This is usually not true for most wild animals and surveillance for disease is opportunistic at best. It is probable that an introduced disease will have spread widely in wildlife before being detected (although the first recognition of highly pathogenic avian influenza in several countries has been in dead waterfowl recently, there is no way of knowing how many cases occurred prior to recognition). Bacon (1981) studied the possible introduction of rabies into the fox population of Britain. The most likely source of infection was thought to be a rabid domestic pet smuggled into Britain, with subsequent spread to foxes, as occurred in Spain in 1975 (Baer 1984). Based on the rate at which cases of rabies in foxes were reported in Europe, together with information on the rate of geographical spread of the disease, Bacon (1981) calculated that rabies might be present for 4 to 7 months prior to being recognized in foxes. During this time, it might have spread radially for 5 to 35 km and involved 100–200 undetected cases. When the index case was detected, authorities would not know where the disease had started and could only guess at an appropriate size and direction for a control zone. Hone and Pech (1990) modeled the probability of detecting foot-and-mouth disease introduced into feral pigs in Australia, with even less comforting results. They concluded that with a density of 15 pigs/km^2 and with opportunistic sampling to detect sick animals "*2002 cases of FMD are likely to have occurred before the outbreak is first noticed about seven months after the outbreak started*". Use of recreational hunters to collect serum from feral pigs for serological surveillance to detect exotic diseases in Australia was judged to be unsuccessful because of poor participation (Mason and Fleming 1999). Enhanced surveillance was an important part of an emergency response plan for dealing with anthrax outbreaks in bison (Government of the Northwest Territories 1999) and is a major factor in the current global concern about H5N1 avian influenza.

The need for rapid action in outbreaks of exotic disease cannot be overemphasized. In the 2001 foot-and-mouth disease outbreak in Great Britain, the policy was to cull all animals from infected farms within 24 h of discovery and from contiguous farms within 48 h (Ferguson et al. 2001). A rapid, appropriate response of this type cannot be accomplished without intensive advance planning involving all relevant agencies. In planning, administrative factors, such as jurisdiction, responsibilities, lines of communication and command, and financial aspects, as well as the actual methods to be used in a potential control program must be addressed. Planning must extend across the entire gamut of anticipated problems, from clarifying who has ultimate authority for various actions, where the necessary diagnostic work will be done, who will report results to the media, to details such as acceptable disinfectants, suppliers of protective clothing, and the method to be used for carcass disposal. Jurisdiction and responsibility become even more complex when wild as well as domestic animals are involved. For example, when we diagnosed Newcastle disease in double-crested cormorants in Saskatchewan during 1990, our diagnostic laboratory was part of a university, Agriculture Canada was involved because this is a 'named' disease for which they have responsibility, the provincial Department of Agriculture was involved because of concern about spread to poultry, the provincial wildlife agency was involved because of responsibility for management of cormorants, the Canadian Wildlife Service was involved because of concern for migratory waterfowl, and the Canadian Park Service was involved because one outbreak was within a national park. Fortunately, in this situation, no emergency disease control was necessary! Many countries have undertaken detailed contingency planning for dealing with introduced diseases of livestock and simulated disease control operations are staged to test and refine the techniques and to maintain a general state of readiness. Wildlife agencies must be an integral part of detailed planning for management of foreign animal diseases. Some agencies have developed detailed emergency response plans to deal with disease outbreaks confined to wild animals, such as anthrax outbreaks in wild bison (Government of the Northwest Territories 1999). In the latter case, the objective is to limit the extent of an outbreak rather than to eliminate the disease.

16.1.1 Are wild animals involved?

Many outbreaks of exotic infectious disease that could potentially infect both domestic and wild animals will first be detected in domestic animals. Shortly after the diagnosis is confirmed, the question will arise as to whether or not wild animals are involved. There will be an urgent need to know: (i) the species of wild animals in the immediate area, (ii) the susceptibility of each species to the disease, (iii) the effect of the disease on the susceptible species (i.e., will they act as maintenance, spillover or dead-end hosts? How easy will it be to identify infected individuals?), (iv) the ability to sample these species,

and (v) the methods available for diagnosis of the disease in the wild animals. An important part of contingency planning for dealing with exotic diseases of livestock is to have as much information of this type readily available, even though there will be many areas of uncertainty. If foot-and-mouth disease were to be diagnosed in my locale, it would be nice to have some basic reference material available, such as that there are 13 species of artiodactyls in Canada, of which seven are known to have been infected with foot-and-mouth disease and the remainder are probably susceptible. More locally, there are seven wild species in Saskatchewan that are likely susceptible and at least one wild species, the white-tailed deer, can act as a maintenance host with long-term shedding of virus. Based on experience with wild animals elsewhere, it is likely that foot-and-mouth disease will not be lethal or visible from a distance in these species. Other information unique to the site, such as the population density and location of susceptible wild animals in relation to infected livestock, and habits and movement patterns of the animals, will be required to assess the risks related to involvement of wild animals in the disease. Some of the latter information can be provided by a biologist knowledgeable about the area. While it is unlikely that all of this information will be available before an outbreak, advance planning should include identifying the individuals and agencies that have this type of knowledge. It is important to realize that initially much of the information, on the basis of which decisions must be made, will be of the best-guess variety. At some point, very early in the program, a decision will have to be made among three options:

- assume that wild animals are involved and implement management procedures without confirmation that they are involved.
- begin sampling to determine if wild animals are involved.
- assume that wild animals are not involved or significant and that nothing needs to done about them.

In some situations it may be critical to undertake sampling to determine whether or not wild animals are involved. In other situations, the first two options may be combined, with management in the form of depopulation proceeding centrifugally from the ‘epicenter’. The animals that are culled can serve as a sample for laboratory examination (obviously, the culled animals must be recovered for laboratory examination for this method to be effective). In this way, management can begin without delay and continue until one is confident that the wild species is not involved or until the disease has been eliminated.

Some general factors must be considered if sampling is to be done to determine if wild animals are involved, these include that:

- sample size is critical, because it influences the probability of detecting disease. A negative result on a sample does not guarantee freedom from disease, but the results from a sample can be used to calculate the maximum

prevalence of disease that could be detected by the sample and, hence, to estimate the risk (see Chap. 7).
- the risk of dispersal of infectious animals from the area as a result of the sampling procedure.
- the time required for collection and analysis of the sample.
- the risk of spreading the infectious agent from the area by the vehicles and personnel used for sample collection.

When Newcastle disease occurred in domestic poultry in southern California in 1972, there was widespread conjecture that the disease had spread to wild birds and that they would disseminate it throughout the continent, rendering any control program in domestic birds futile (Hayes 1980). Rapidly organized, massive sampling of free-flying birds identified only four infected individuals among 9,466 specimens examined. The infected birds were house sparrows and a crow, all directly associated with infected poultry. This was taken as evidence that the disease had not become established in wild birds. Based on this information, the eradication program proceeded by concentrating on control of the disease in domestic birds and was successful in eliminating the disease.

16.1.2 Management of the outbreak

The actual procedures to be used in the event of diagnosis of a foreign animal disease depend to a large degree on the features of the disease involved. Foot-and-mouth disease is often used as the example for such programs, and I have used it here because of the amount of information that is available. However, in many ways, foot-and-mouth disease is a worst-case scenario because: (i) of the breadth of species that can be infected, (ii) its exceptional transmissibility, and (iii) animals commonly become infectious before clinical signs appear. The need for haste and extreme measures may be less in some other diseases. The other major factor that determines the course of action is the size of area involved. Most emergency control programs are designed to deal with relatively localized events. If a disease has become established over a very large area, procedures described in earlier chapters, that have a more gradual effect, will likely have to be applied.

If the disease is in livestock and has not reached susceptible wild animals, every effort must be made at the local level to prevent contact between infected domestic stock and wild animals. **Biosecurity on intensive livestock operations, such as poultry and hog barns, is usually designed to prevent the entry rather than the exit of disease agents.** In the event of an emergency situation, this must be extended to include 'reverse biosecurity', i.e., security to prevent wild animals having direct contact with domestic animals or with infectious effluent from domestic animals. This means controlling carcasses, manure, water, and air leaving the facility. Where there is a high probability

of contact, as might occur between cattle and deer on pasture, emphasis must be placed on preventing further contact, such as by removing the cattle, while at the same time preventing dispersion of potentially exposed wild animals away from the immediate area. The latter might be done through the use of attractants, fencing, or by local depopulation in a manner that will not cause dispersion, such as by trapping animals or killing animals with poison baits. As an example, contingency planning for the possible introduction of rabies into the fox population of the United Kingdom includes fox depopulation using poison baits (Smith and Wilkinson 2003).

Disease management in the event of a localized outbreak usually consists of establishment of a series of concentric zones surrounding the recognized point(s) of infection. Within the concentric zones (Fig. 16.1) the movement of animals and people is rigidly controlled; animals and premises are inspected regularly by veterinarians for evidence of disease, infected and exposed animals are slaughtered and disposed of in a prescribed manner, infected premises are decontaminated, and owners are compensated for their losses. In some cases, vaccination may be used either to immunize contacts, or to immunize all individuals in close proximity ('ring vaccination'). The severity of the measures applied decreases with distance from the epicenter. An important general principle is control of the **direction** of movement of animals and potentially contaminated material. **Movement into the infected area is minimized** and **movement out of the area is prevented.** In the 2001 outbreak of foot-and-mouth disease in Great Britain, movement bans were credited with preventing almost all long distance and most short range

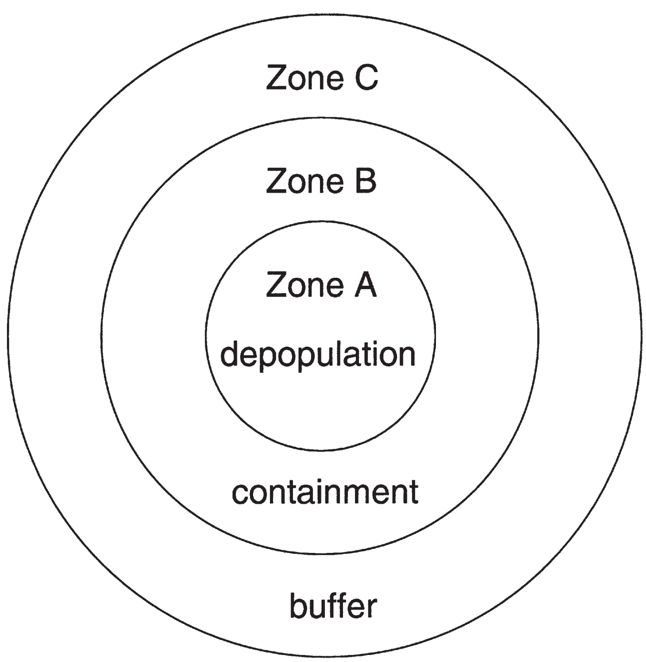

Fig. 16.1 An example of the type of radial zones that might be established around a focus of exotic disease. The rationale is to reduce movement of animals into the central area and to prevent any movement of animals or infectious material outward

movement of livestock, and with reducing the rate and range of disease transmission (Ferguson et al. 2001). Management of an outbreak of foot-and-mouth disease in cattle in Zimbabwe (Brückner et al. 2002) is an excellent example of this process, including the need to expand the zones as new cases are recognized.

The same general procedure is likely to be applied to wild animals, with the recognition that the animals cannot be observed or inspected readily, that bans on movement or legal quarantines have no effect, and that it is impossible to trace contacts (individuals that have been in contact with an infectious individual) in the manner used in management of human and livestock diseases. These limitations mean that all wild animals, of susceptible species, in the control area have to be treated as though they were infected or exposed and that actual, rather than paper, barriers are required to stop movement. The width of the control zone will depend on the rate of spread of the disease and the mobility and range of the species involved. Obviously, a wider control zone is required for highly mobile species, such as the coyote, than for more sedentary species, such as the skunk. This requires knowledge of the normal home range and movement patterns of the species involved. In the zone immediately about the point of infection, the population of wild animals should be reduced in a manner that does not cause dispersion, e.g., by poisoning or trapping (techniques such as poisoning may limit the ability to collect animals for laboratory examination). The objective in the next zone is to deter entry of low-risk wild animals into the zone from the outside and to contain high-risk animals from the inner zone so that they do not disperse. Methods might include fencing, scaring devices and regular patrols, or the animals could be immunized. The peripheral zone acts as a further buffer between infected and non/infected areas. Contact between domestic animals and wild animals should be minimized, and activities, such as hunting, which might disperse animals, should be prohibited in this zone. Radial zones of this type were used in response to incursions of raccoon rabies into Ontario. Raccoons and striped skunks captured by live-trapping within a 5-km radius of the site were euthanized, raccoons and skunks trapped within 5 to 10-km radius of the site were vaccinated and released, and vaccine baits were spread by aircraft over a large peripheral zone (Rosatte et al. 2001). Based on a predictive model, Smith and Wilkinson (2003) suggested that culling about the disease focus, together with an outer ring of immunization, or immunization plus fertility control could be the best strategy to control a localized rabies incursion in foxes. Concentric rings with restrictions on movement of poultry from the inner zone and isolation of domestic birds from contact with wild birds were instituted when highly pathogenic H5N1 avian influenza virus was detected in a wild whooper swan in Scotland (Defra 2006).

This general technique for disease containment and eradication has been tested and refined in the control of outbreaks of disease among domestic livestock in many countries but its efficacy in the event of an exotic disease becoming established in wild animals is largely untested. Massive depopulation

of deer was used in the control of an outbreak of foot-and-mouth disease in California early in the century and more than 20,000 deer were killed in Florida as part of the cattle fever tick eradication program (Hayes 1980). Hundreds of foxes were killed in Spain following a focal introduction of rabies in 1975 and this was apparently successful in preventing an epizootic (Baer 1985). Smith and Wilkinson (2003) modeled the relative efficacy of culling through poison baits, oral vaccination, and vaccination plus fertility control in eradicating an epizootic of rabies in foxes in the United Kingdom.

Reports dealing with focal eradication of feral pigs in Australia provide insight into the type of program that might be needed if a foreign animal disease became established in large ungulates. Hone and Bryant (1981) described an eradication program for feral pigs that might be used on a 200-km^2 area centered about a hypothetical foot-and-mouth disease outbreak in cattle. The area for this hypothetical exercise was bounded on two sides by water, so that control would be more difficult on areas with no such natural barrier. Two zones were proposed: a central zone within which all pigs would be eliminated and an outer 1-km-wide perimeter zone. Control would start in the perimeter zone and it was estimated that pigs could be eliminated in this zone within 3 days by a combination of shooting from five helicopters and trapping. At the same time that pig eradication was proceeding, the two sides of the area not bounded by water would be enclosed by pig-proof fencing. It was estimated that it might take at least 6 days to complete the 30-km fence, using 30 men, and that a shortage of fencing material might "*inhibit*" construction (maintenance of a list of sources for supplies, such as fencing, disinfectants, and bulldozers must be part of the contingency planning process for dealing with introduced diseases). Fencing was considered unnecessary if the density of pigs was low in the area. Following successful eradication in the perimeter zone, this area would be patrolled by helicopter by day and by road at night to prevent repopulation. The main method proposed for eradication of pigs in the internal zone was shooting from helicopters. Trapping, hunting with dogs and from boats, and burning of vegetation also might be used. It was estimated that eradication would require use of six helicopters for 20 days if the density of pigs was 8/km^2, and could be completed in 6 days if the density was 2 pigs/km^2. Carcasses of pigs killed by shooting would be collected for disposal. The estimated total cost (in 1981) of from \$48,000 to \$173,000 (Australian), depending on the density of pigs, was considered "*insignificant compared to the expected economic benefits of FMD eradication*".

Hone (1983) described an actual attempt to eradicate feral pigs on a 50-km^2 area. The regions adjacent to two sides of this area contained no pigs. The major methods used for eliminating pigs were poisoning with sodium fluoroacetate (1080) followed by shooting from helicopters. The population of pigs on the area was unknown (as is usually the case with wild animals); however, the relative density of pigs before and after control was measured. Pigs were baited with poison-free feed for 9 days prior to placement of the poison (obviously, this type of prebaiting would not be possible in an actual

emergency). It was estimated that 73% of the pigs on the area died as a result of poisoning. After poisoning was completed, 98 live pigs were seen from a helicopter; of these 95 were shot and three escaped. The conclusion was that the methods used rapidly reduced the abundance of feral pigs and that eradication "*would be almost complete but probably not entirely so*". It was thought that "*less time and more money would be available*" in a real disease emergency and that containment of pigs to the area during the control program and prevention of recolonization would need to be addressed. Only 17 pigs killed by poison were recovered, and many carcasses remained on the area. These could act as a potential source of contamination (and possible spread of the disease by scavengers not killed by 1080) and few specimens would have been available for examination to determine the rate of infection in pigs.

Saunders and Bryant (1998) evaluated the effectiveness of a plan to eradicate feral pigs on a 120-km^2 site in New South Wales. Of the estimated 1,238 pigs on the area, 80% were removed; 946 by shooting from a helicopter and 43 by trapping or shooting on the ground. Radiotelemetry indicated that some pigs learned to avoid detection from helicopters. It was concluded that eradication of feral pigs for control of exotic disease "*may be an unrealistic goal*" and isolation and containment of pigs may be more efficient.

These examples, together with a report by Ridpath and Waithman (1988) describing an attempt to eliminate feral water buffalo from a 389-km^2 area in Australia, and a further modeling exercise on foot-and-mouth disease in feral pigs (Pech and Hone 1988), serve to illustrate the massive amount of equipment, manpower and effort that is required for an emergency eradication program, even on a relatively small area. The amount of money required for a major program is likely to be far beyond the normal budget of a single agency. In considering the examples dealing with feral pigs, it must be remembered that: (i) the involved areas were only 200 and 50 km^2, (or circles with a radius of <8 and <4 km), respectively, and (ii) in both cases, there were natural barriers on two sides of the study area that aided the depopulation program. By comparison, Bacon (1981) estimated that fox rabies might have spread over a radius of 35 km (or an area of >3,800 km^2) prior to detection and Hone and Pech (1990) estimated that spread of foot-and-mouth disease among feral pigs "*into adjacent areas could result in outbreaks covering up to 10,000 or 30,000 km^2*" by the time of first diagnosis. During the most recent foot-and-mouth disease outbreak in North America, (Saskatchewan 1951–52), 21 rural municipalities were included within the quarantine zone (Wells 1952) and the straight line distance between the two most distant infected farms was >105 km. Fortunately, this outbreak occurred during winter when all cattle were stabled, so that the disease did not spread to wild deer.

Public reaction to any potential control program must be considered carefully in the planning process. Feral pigs are considered a nuisance in Australia but have considerable economic value in some other areas and "*there are many socio-economic factors associated with feral swine that must*

be carefully evaluated for eradication to be successful" in areas such as Florida (Degner et al. 1983). There likely will be vigorous public reaction to the thought of helicopter gunships and poisons being used, particularly if popular species such as deer are involved. There may be great difficulty in convincing groups such as hunters of the need for slaughter of wildlife, particularly if they perceive only a loss of their resource with no personal gain. Under such circumstances, one should anticipate the probability of court challenges or injunctions to stop or delay the control program, in the same way that court challenges were used to block an emergency deer hunt in Florida (Florida Game and Fresh Water Fish Commission 1983). Massive public education must be a very important part of any control program that is deemed essential.

A final factor that must be considered in any program that involves the emergency killing of large numbers of animals is how the carcasses will be disposed of. Because the animals are potentially infectious, the carcasses should not be moved from the site. However, environmental legislation to protect ground water and air have made procedures that were routine in the past, such as burying or open pyre or pit burning on site, increasingly difficult. Although the scale of any wild animal depopulation will never reach the level of carcass disposal that occurred during the 2001 foot-and-mouth disease outbreak in Great Britain, the problems and solutions discussed by Scudamore et al. (2002) are instructive.

It should be obvious from the above discussion that emergency control is only likely to be effective in controlling an introduced disease when the disease is detected shortly after arrival and is localized to a small area. This stresses the importance of strict measures to prevent introduction of foreign diseases, the need for regular surveillance and diagnosis of diseases in wildlife for early detection, and the need for contingency planning for dealing with emergency situations. In some circumstances, even in domestic species, quarantine and eradication may be inadequate for dealing with an incursion of a foreign disease. This is particularly true when the disease has gone undetected for some time and has spread over a large geographic area. Even with diseases such as foot-and-mouth disease, it may be necessary to fall back to alternative plans, such as containment by vaccination, despite the massive economic penalties a delay in elimination of the disease might entail (Krystynak and Charlebois 1987). However, this type of program presupposes that sufficient quantities of an appropriate vaccine would be available on very short notice and that methods for delivering vaccine to wildlife are available (neither of which is likely to be true). Ring or barrier vaccination (assuming a suitable vaccine and method of delivery are available), might be an alternative or an adjunct to radial depopulation for the control of foci of disease in areas that are otherwise free of the disease. Animals in a perimeter zone might be immunized, while those in the central core area were eliminated, as was done to deal with raccoon rabies in Ontario. The presence of an established population of immune animals in the periphery should help to reduce influx of susceptible animals into the depopulated core area and spread of disease from the core outwards.

Yekutiel (1980) described four phases to a disease eradication program and these are applicable to emergency management programs. The first phase consists of training personnel, assessing the resources needed and available, enumeration of the population of concern, and establishment of the necessary administrative structure. This phase should have been completed in advance, as a part of the contingency planning process. Phase 2 consists of implementation of the actual management operation and there must be "*meticulous execution of the control measures*" and "*strict completion of work schedules in place and time*". This phase continues until the prevalence of the disease has been reduced to a level where further transmission is unlikely to occur. This point will be different for each disease, and a very important consideration is whether or not recovered animals remain as infectious carriers. The third phase is a mopping-up operation in which there must be intense surveillance to detect any remaining cases and tracing of all infected cases to ensure that all contacts are detected. As noted earlier, trace-back of contacts that is used commonly in both human and veterinary medicine is impossible in wild animals. Problems often occur in phase 3 of disease eradication schemes in humans because of difficulties in detecting the few remaining cases, particularly where there is a "*paucity of basic health services*". This can be anticipated to be the normal situation in wildlife. Hone (1983) expressed a similar thought, in that it would be "*difficult to locate and kill the last few pigs in an area and this appears to be the major obstacle in the execution of contingency plans for the eradication of feral pigs to control exotic disease. A further difficulty is to know when feral pigs have been eradicated, as a survey result of zero is ambiguous*". The final phase in an eradication program involves steps to prevent reintroduction of the disease to the area.

In reviewing emergency disease control in wild animals, four points have become evident to me. The first is that the system of contingency planning and the methods used for the control of exotic diseases in livestock form a good theoretical base for similar programs in wild animals. However, the actual methods for implementing these plans are largely undeveloped and untested. It is a useful mental exercise to contemplate the methods that would be most appropriate and how much time would be required, if it became necessary to eliminate a dense population of deer in a 500-km^2 forested area. The second point is that total eradication of a potentially exposed population, as is done in domestic animals, is probably impossible, at least in the short-term, in most wild species. The goal in such situations has to be containment of the disease to an area and reduction of the population density within the area to a point where disease transmission ceases and the disease dies out. However, population reduction in an area "*may be counter-productive if the subsequent contact rate is increased because of immigration of susceptible animals*" (Pech and Hone 1988). The third point is that fall-back measures, such as vaccination, have received almost no attention in wildlife and generally would not be available for use. The final thought is that we, in North America, have been extremely fortunate that the foreign animal diseases that have

reached our shores have, in general, not become established in wild animals. Outbreaks of foot-and-mouth disease in Mexico in 1926 and 1946 and in Canada in 1952, Newcastle disease in California in 1972, and pathogenic avian influenza in Pennsylvania in 1983, were eradicated by measures restricted to domestic animals, without the necessity of any control in wild animals. Similarly, foot-and-mouth disease was eliminated in Great Britain without the need for management of wild animals, although all five deer species present are susceptible to the disease (Gibbs et al. 1975). In most of these cases it appears to have been good fortune, rather than good management, that disease did not spread to wildlife.

16.2 Integrated disease management

The goal of disease-management programs, particularly those designed to prevent or control disease, must be sustainability. Examples have been cited in earlier chapters of the risk inherent in relying on a single method for the management of any disease, particularly over the long term. The concept of integrated management has gained (or perhaps more correctly regained) favor in public health as overly optimistic projections that disease could be eliminated by a single method, such as the elimination of vectors with insecticide or mass treatment of infected individuals, have failed to produce the desired result. As stated by Gubler (2001), "*failures occurred when we became complacent after successes were achieved and relied too much on the "quick fix" or the "magic bullet approach to disease control*". The concept of integrated disease and pest management is well established in agriculture. There the goal is to use a variety of different strategies to maintain disease and pests at a level at which there is minimal economic damage, while conserving environmental quality. One effect in agriculture has been a reduction in the overall use of agrochemicals.

There are four components to an integrated management program: (i) detailed knowledge of the disease, (ii) monitoring of the disease or some indicator of disease, such as insect vector population to detect when management is necessary, (iii) implementation of the most appropriate management strategy based on surveillance and the particular situation, and (iv) education.

In agricultural use, integrated disease or pest management depends on cost/benefit ratios to make decisions about when management is required and what type of management is most advantageous. Usually an economic threshold can be identified at which management action should be taken to prevent an increasing disease incidence or pest population from doing economic damage. This approach does not apply to many diseases of wildlife because it is not possible to estimate a dollar value of the benefit for comparison with the cost of action, except perhaps where a disease in wild animals

such as plague might interfere with tourism in an area or where a disease is shared by wild animals and livestock. For most diseases, it is more appropriate to consider cost-efficiency analysis, the purpose of which is to allocate the resources available as efficiently as possible (Ginsberg 2001). As an example, Selhorst et al. (2001) examined the cost-efficiency of various baiting strategies for vaccination of foxes against rabies.

Programs to manage disease must be well designed and sufficiently flexible to change with evolving conditions as the program proceeds. Various techniques to deal with agents, hosts, habitat conditions, and humans have been considered individually in the previous chapters but, in practice, a combination of several methods may be required or be more efficient than any single technique. Modelling may play an important role in integrated management by providing a method for predicting the probable success of different methods under varying circumstances. In an early example, Anderson et al. (1981) used a model to suggest that a combination of vaccination and culling to reduce the population density of foxes would be more cost-effective than either strategy alone for the control of rabies, "*as well as permitting more foxes to live than does pure culling*". Selhorst et al. (2001) modelled different vaccination strategies for rabies in foxes to determine the optimal time for bait distribution. Smith and Wilkinson (2003) used a predictive model to evaluate the relative efficacy of culling, oral vaccination, and vaccination plus fertility control to control fox rabies. They found that the probability of success of the various techniques varied with the density of foxes. Swinton et al. (1997) and Gormley and Collins (2000) compared various methods for control of tuberculosis in badgers. Hayes et al. (1999) modelled the effectiveness of various interventions including use of acaricides, reduction of deer density and exclusion of deer by fences, and tick habitat modification for preventing Lyme disease in humans.

In an integrated program, different methods may be used simultaneously against various parts of the transmission cycle of a disease, or a series of techniques may be employed sequentially, as the overall program proceeds. Various potential measures can be divided using two sets of criteria. The first separation is between those measures having a short-term effect and measures that have long-term effects. The second division is between measures that are specific to the control of a single disease and other measures that are non-specific but which improve the general level of health of the population and indirectly reduce the prevalence or intensity of the disease. In general, short-term specific measures may result in a rapid reduction in the prevalence or intensity of a disease but are often costly, transient in their effect, and may have little impact on the more general health of the population. Unfortunately, many agencies prefer to fund quick-fix approaches, because it gives them greater visibility (Gubler 2001). In the same situation, a long-term, non-specific change, such as improved habitat, may have a less dramatic effect on the immediate problem but may reduce the occurrence of the disease over time, as well as having other beneficial effects for the population.

The costs of habitat manipulation could be shared among several benefits and spread over a period of years.

An integrated disease-management program may contain both short-term and long-term measures. For instance, the long-term specific solution to lead poisoning of wild birds worldwide is total cessation of use of lead shot for hunting, lead sinkers for fishing, and elimination of other sources such as mine wastes rich in lead. However, until that can be achieved, a number of short-term measures may be necessary. In parts of North America these included local bans on the use of lead shot on specific wetlands and lead sinkers in certain waters, and local measures to prevent birds using contaminated wetlands. Public education promoted voluntary use of non-toxic shot and encouraged compliance with bans on lead shot use. Even when the use of lead shot and sinkers ceases, the problem will continue for some time because of the massive number of pellets that occur in the soil of many marshes. Lead poisoning will continue to occur on these sites, although at a reduced rate. The lead will become less available over time but short-term techniques, such as cultivation of the soil, might hasten the process. Birds also might be discouraged from using high risk areas until shot or sediment is no longer readily available. Similarly, in some problems such as pneumonia in bighorn sheep, short-term measures, e.g., treatment with anthelmintics or antibiotics may be required in the interim, until changes in habitat and population distribution can be affected. In such cases, the short-term specific measure often is used to buy time. It is important to remember that short-term measures often are equivalent to the symptomatic treatment of a patient with an analgesic. In both cases, the treatment may relieve the immediate pain without curing the underlying disease. In some instances, palliative treatment may disguise the fact that the situation is actually worsening and hinder implementation of more appropriate techniques.

A combination of methods may be used in other ways. For example, in managing bovine tuberculosis in wild cervids, such as in white-tailed deer in Michigan or elk and deer in the area of Riding Mountain National Park in Manitoba, there is a need to reduce contact between cervids and cattle, reduce factors that concentrate cervids and enhance transmission, and to reduce or maintain cervid populations at a level at which disease transmission may be reduced. The methods used have included assisting livestock owners to fence supplies of stored hay to prevent use by cervids and contact with cattle, prohibiting intentional feeding and baiting of cervids, encouragement of enhanced hunting to reduce populations, and preferential culling of diseased individuals. Strategies proposed to control *Echinococcus multilocularis* in areas where it is transmitted mainly by foxes include public education campaigns to inform the public about the disease and to minimize contact between people and foxes, restricting access of pet dogs and cats to rodents, and chemotherapy of foxes (Ito et al. 2003).

It may be appropriate to use different techniques to accomplish specific goals at different times within a management campaign. The program to

eradicate brucellosis from domestic cattle in Canada provides an example of such a phased approach. The concentrated effort toward eradication began in 1950, at which time the national infection rate (prevalence) was about 9%. The initial approach was institution of a uniform, country-wide, program of vaccination of calves. It was recognized in advance that this would not result in eradication of brucellosis. The objective of vaccination was to reduce the overall prevalence to a level at which it would be economically acceptable to use other means. Immunization was successful in lowering the prevalence rate to 4.5% by 1956, at which time it was considered possible to begin selective depopulation (test and slaughter). Mandatory farm-by-farm testing was begun in 1957. Infected herds were quarantined and animals that reacted positively to the test were slaughtered, with compensation being paid to owners. As the prevalence of the disease declined further as a result of these measures, new methods of surveillance were introduced that reduced the amount of on-farm testing required. These included testing of cattle and of milk going to market, with trace-back of animals that reacted positively to the farm of origin. Vaccination was de-emphasized, partially because titres (as a result of vaccination) interfered with surveillance testing. More rigorous quarantine measures were introduced and strict controls were placed on the movement of cattle to reduce spread of the disease from areas that were still infected to brucellosis-free areas. When the prevalence of the disease became very low, more sensitive surveillance methods were required and the emphasis shifted to total depopulation of infected herds, rather than selective removal of test-positive animals. The overall program was successful and Canada was declared officially free of brucellosis in 1986. The emphasis shifted to preventing reintroduction of the disease and to dealing with the last remaining reservoir of the disease in wild bison in and around Wood Buffalo National Park.

Public education is often combined with other techniques in integrated disease-management programs and may serve a very valuable function, not only in demonstrating the need for the program but also in changing human activities to reduce the effects of the disease. For example, a major objective of most attempts to control rabies is reduction of the risk of human exposure to this disease. An integrated management program for rabies might include public education regarding the risks of approaching or handling wild animals that act abnormally, a campaign to promote regular vaccination of pets to reduce the risk of these animals acting as an intermediary between infected wild animals and humans, control of stray dogs and cats, and oral vaccination of wild hosts. This can be supplemented by emergency procedures such as local depopulation of the principal wild animal vector. Various combinations of these factors have been used in different parts of the world, with public education being an integral part of almost all campaigns.

The most important step in the process of planning an integrated management campaign is a careful analysis of the objectives of the program and of all the possible methods for achieving these goals. Each potential method will have strengths and weaknesses but when these are listed the most appropriate

techniques for the situation can be chosen. The progress of the campaign must be monitored constantly once it is underway and methods changed as conditions alter.

16.3 Summary

- Emergency measures are usually only required for occurrences of new or exotic diseases.
- Containment, followed by eradication, is the method of choice for dealing with focal disease emergencies.
- The success of an emergency disease-management program depends on early detection of the disease and rapid implementation of management measures. The latter requires extensive prior planning and testing of the system.
- Concentric quarantine zones are usually established about foci of disease to minimize animal movement into the area and prevent movement out of the area. This may involve radial depopulation and construction of physical barriers, such as fences.
- Alternate or fall-back strategies, such as immunization, must be planned in the event that the disease cannot be contained.
- Methods used for emergency control of exotic diseases of livestock may be generally appropriate for wildlife but these have not been tested or refined through actual field application.
- Management programs that rely on a single technique often fail because of changes in the disease, the host, or the environment.
- Once the objectives of a program have been defined, all possible solutions should be evaluated singly and in combination.
- Short-term, specific measures often result in a rapid decrease in disease occurrence, but are usually expensive, transient in their effect, and may have little impact on the general health of the population.
- Long-term, non-specific measures may reduce the occurrence of disease over time, as well as having other beneficial effects on the population.
- Combinations of short and long-term measures, applied either simultaneously or consecutively, are often more effective than single methods.
- Public education should be included in every integrated disease-management program.
- Continuous monitoring of the program is necessary so that new methods can be introduced as current methods become ineffective.

17 Assessing the effectiveness of a disease-management program

"*The ultimate practical aim of epidemiological investigations is to establish effective disease control measures, whose effect in turn should be evaluated by epidemiological methods in order to ensure that the highest health benefits are derived from available resources and techniques*"

(Cvjetanovic 1976)

Management or control of disease in humans, domestic livestock, or a wild animal population is a costly and controversial exercise. For these reasons, it is imperative that the effectiveness of programs must be scrutinized carefully, so that the scarce resources available are allocated rationally and no unnecessary action is taken. **It is fair to characterize most methods currently in use for disease management in wild animals as being of unproven and untested efficacy.** Even such a basic technique as the collection and disposal of carcasses during epizootics has not been tested adequately to determine if: (i) a significant proportion of the carcasses present are collected, (ii) carcass disposal has a significant effect on the outcome of an outbreak and, (iii) the same resources committed to other methods might be equally, or more, effective. Some such techniques seem intuitively correct and may be used in part because "*an action program is appealing to the public*" (Davis 1974); however, intuition and public appeal should not be viewed as a substitute for careful objective appraisal. When the effectiveness of carcass collection and removal was tested during botulism outbreaks in western Canada, it was found that this labor-intensive management practice had no significant effect on the mortality rate among marked mallards (Evelsizer 2002). If this finding is more generally applicable remains to be tested. Reliance on untested methods may result in wasted effort that might have been used more productively, it may hinder the development of new methods that are more effective, and it may allow the progression of a disease to a point where it becomes unmanageable. One must always be cognizant that learning from experience is not the same as making a mistake repeatedly until you are good at it!

Before any disease-management program is begun, the participants should decide upon the methods that they will use to assess its effectiveness. The steps in the planning process for a project might be characterized as deciding that:

- this what we want to do (objectives)
- this is how we will do it (methods)
- this is how we will know if it is working (assessment of effectiveness)

Every disease-management program should have clearly defined objectives established in advance. Once an objective has been identified, it then provides a standard against which the effectiveness of the program can be measured. For example, assume that the objective of a control program is to reduce the prevalence of disease "X" from an unacceptable 7% to a tolerable 2%. The effectiveness of this program can be measured quantitatively and it is evident that one could establish intermediate goals with timelines. One might decide that a reduction in the prevalence rate by 1% in each of the following 5 years would be acceptable performance. In planning how an assessment will be done, a number of major factors, including the parameters to be measured, the methods of collecting the data, the methods for assessing the information, and the time frame must be considered. These will be discussed individually.

17.1 Choosing suitable parameters to measure

The success of a disease-management project might be measured in a variety of ways. In selecting parameters for measurement, one should choose those that are directly meaningful to the objectives of the project. For example, when Schmidt et al. (1979) evaluated the efficacy of drug treatment of lungworms in bighorn sheep, the parameter chosen for measurement was the survival rate of lambs. The objective of the project was to improve lamb survival by preventing pneumonia, so that survival rate was the parameter of most direct meaning to the project, the sheep, and the biologists. Similarly, if one was trying to measure the effectiveness of carcass collection as a technique for reducing losses of waterfowl to botulism or avian cholera, measuring the mortality rate of marked birds on wetlands with and without carcass collection would give the most direct evaluation of the effectiveness of the technique. Counting the number of carcasses collected during a management program does not measure the effectiveness of the technique (unless you can relate this to the population at risk and calculate the mortality rate). Measuring the proportion of carcasses present in the marsh that is found and collected is not a measure of the effectiveness of the method but this information would be useful in understanding why the technique is or is not effective.

It is important to choose parameters for which detection tools of known effectiveness are available. One would not choose to use the prevalence of antibody titres as a parameter for measuring the efficacy of an immunization program, unless a suitable serologic test of high specificity and sensitivity was available. As an example, assume that the objective of a program is to protect

animals from a disease through oral immunization using a bait system. In assessing the effectiveness of the program one might measure any or all of the following:

- the proportion of the population that consumed one or more baits (this could be done by including a marker, such as tetracycline that marks bones and teeth, in the bait).
- the proportion of the population that had detectable antibodies after placement of baits.
- the proportion of the population protected against the infection.
- the actual occurrence of the disease (as measured by the prevalence or incidence rate) in the population after baiting, in comparison to that before baiting.

In this example, the most meaningful measures would be the proportion of the population that was protected by the vaccine and any change in the frequency of occurrence of the disease attributable to immunization. However, it usually is difficult or impossible to measure the actual degree of protection that occurs in the field, because of the difficulty in determining the rate and manner in which animals are exposed to the disease. For this reason, other parameters that are more easily measured, such as the proportion of animals that consumed bait or the prevalence of antibodies, might be used. It is important to recognize that these are indirect measures. The proportion of animals that consumed a bait might not be a good indicator of protection, if some of these animals fail to become immunized. Even antibody titre may be a poor predictor of actual protection in the field. Experimental studies to determine the proportion of the animals that are marked after consuming a bait, and the relationship among the consumption of baits, development of antibody titres, and protection against experimental challenge would have to be done to establish the effectiveness of these indirect parameters as indicators of the desired effect.

Changes in indices of infection or in rate of occurrence of disease in the population are the most direct measures of the effectiveness of a management program but this type of information may be very difficult to collect in wild animals. Both prevalence and incidence rates are measures of the occurrence of disease within a population and either could be used to assess the efficacy of a control program. However, it is important to recall that incidence is a measure of the number of new cases of disease that occur in the population during a period of time, while prevalence is a measure of the cases existing in the population at a specific time. Incidence rate is usually the better parameter for assessment of a control program because it is a direct measure of how well the program is working to prevent animals from becoming diseased. However, prevalence often is used in place of incidence because it is much easier to measure. One can measure the prevalence of antibodies to an agent in a population by conducting a single cross-sectional survey of a population.

Measurement of the incidence of disease in the same population over a time period would require at least two data collections: one at the start of the period to ensure that the animals did not have antibodies and a second in which the same animals are recaptured to determine the proportion that developed antibodies during the observation period.

Prevalence rate is an adequate means of assessing the effectiveness of many management programs but it has limited value for certain types of disease, particularly for: (i) diseases with a high case fatality rate, and (ii) chronic diseases with a protracted course. Tularemia in cottontail rabbits and muskrats is an example of the first of these exceptions. Virtually every animal of these species that becomes infected with *Francisella tularensis* dies shortly thereafter of the disease. Thus, it would be very unusual to detect either a sick animal or an animal with antibodies to this organism during a cross-sectional survey, although many cases actually might be occurring in the population. The incidence rate over a time period in highly fatal diseases such as tularemia usually is much greater than is indicated by the prevalence rate at any time during the period. In contrast, in chronic diseases such as tuberculosis, the duration of individual cases may be so prolonged that the prevalence rate changes slowly, making it difficult to detect changes in the rate at which new cases are occurring. Even if management is completely successful in preventing transmission, prevalence only declines as infected animals die, the infectious agent dies out, and dilution occurs through the entry of uninfected animals to the population. In this type of disease, the prevalence rate may be much greater than the incidence. However, even in chronic diseases, the prevalence rate will decline slowly over time if the management program is effective in preventing the occurrence of new cases and this change might be detectable through repeated cross-sectional surveys.

An indirect method for estimating the rate of transmission of a disease within a group of animals is to examine age-specific prevalence rates. Assume that we wish to measure the effectiveness of a program for controlling a parasitic infection in a deer population. The treatment used acts by preventing infection but it has no effect on established infections of the long-lived parasite. The only specimens available for examination are hunter-killed animals in autumn. This sample provides a cross-sectional view of the population once each year. The prevalence of the parasite in 6-month-old fawns during the hunting season provides an estimate of the incidence over a 6-month period, assuming that the animals were born free of the parasite. This rate could be used for comparisons between years to measure the effect of a management program. If annual sampling were done, the infection rate in different age cohorts could be monitored over time (Table 17.1). Note that in this hypothetical example, most animals became infected early in life and then the infection rate remained rather constant in older age groups. Following the start of the control program, the overall prevalence in the total population did not decline markedly in the first year after treatment but the prevalence among fawns declined dramatically. The following autumn the prevalence of

Table 17.1 Changes in the age-specific and overall prevalence rates (%) of a parasite in a hypothetical deer population, in relation to a management program instituted in the spring of 1985. The prevalence rates are based on a sample of specimens collected from hunter-killed deer each autumn

	Age (years)							
	0.5	1.5	2.5	3.5	4.5	5.5	6.5	Overall
1984[a]	80	84	83	85	78	74	72	80.5
1985[b]	6	80	81	80	78	72	71	74.0
1986	4	7	78	80	72	70	71	38.0
1987	7	5	6	75	68	72	65	25.1

[a]Pre-control
[b]6 months after start of control program

the parasite in 1.5-year-old deer provides an estimate of the number of new cases in this cohort over the intervening year, assuming that the animals infected in year 1 remained positive in year 2. One could also examine both the prevalence and intensity of infection of older deer in the population over time as an indicator of the effectiveness of the program. In the absence of re-infection, the parasite burden should decline gradually in both intensity and prevalence. Cohort studies of this type are only applicable for indicating changes in a relatively sedentary population and would not be reliable in populations where there is extensive movement and interchange.

No single parameter may be adequate for the total assessment of a disease-management program and several may be used simultaneously or consecutively. For instance, three indices (possum abundance, prevalence of tuberculosis in possums, and prevalence of tuberculosis in cattle) were used to assess the effectiveness of possum reduction in New Zealand (Coleman et al. 2006). A final criterion for selecting parameters is that they should be simple, inexpensive, and standardized. This is particularly true if there is to be continuous evaluation of the program. As an example, a nationally standardized 'residual trap-catch index' has been adopted for evaluating possum populations in New Zealand (Anonymous 2004).

17.2 Collecting the data

Once suitable parameters have been chosen for assessment, the next step is to decide how the information related to these parameters will be collected. In human medicine, routine health statistics, such as morbidity or mortality rates, may be available and adequate for this purpose. This type of information seldom is available for wild animals. If the disease causes obvious illness or

mortality, one might be tempted to use existing data, such as the number of reported cases or the number of submissions to diagnostic laboratories, as a source of information. This type of information is subject to serious selection bias and may be an unreliable indicator of the actual occurrence of a disease. For example, during the early stages of a disease-management program there is likely to be a high level of public awareness and, consequently, there may be a high rate of reporting and submission of cases. If prevalence of the disease declines, public interest and even the attention given the disease by professionals, such as biologists and conservation officers, are likely to decline as well. This may result in a spuriously low rate of reporting of the disease and an overly optimistic assessment of the efficacy of a control program. We have observed this phenomenon of the relationship between interest in a disease and reporting of diseased animals on several occasions. When sarcoptic mange spread from west to east across Saskatchewan in coyotes during the late 1970s, we received many submissions of affected coyotes from areas along the leading edge of the wave, but very few animals were submitted from the region behind the epizootic front. When questioned about this, conservation officers indicated that the disease was still present and common in these areas but, because everyone 'knew' the cause, there was little interest in submitting specimens to the laboratory. In this instance, the lack of reporting of diseased animals gave the false impression that the disease had disappeared behind the wave, when in fact it had assumed an enzootic form. More recently, we have found that the number of submissions of dead corvids (common crows, blue jays and black-billed magpies) to the laboratory for examination for West Nile virus was high in the first year of a surveillance program, but then declined as people lost interest in the subject. Data collected in an opportunistic manner, such as by using the number of reported cases of a disease, also may be unreliable because of variations in the diagnosis of disease. For example, not all of the coyotes seen to have hair loss in Saskatchewan were suffering from sarcoptic mange, so that statistics using hair loss as a criterion, without laboratory confirmation, would have lead to an overestimation of the occurrence of sarcoptic mange. A further disadvantage of using such data is that is impossible to calculate meaningful rates in relation to the population. For instance, if ten cases of the disease are reported in a year, it is impossible to calculate the actual prevalence rate, as the population from which these animals was drawn is unknown and likely is not constant.

In many circumstances, special surveys or planned surveillance are necessary to collect the information needed for the evaluation of a disease-management program. This usually involves some form of sampling and the methods for sampling and for selecting appropriate sample sizes are the same as those described in earlier chapters for other types of investigation. Consultation with a knowledgeable biometrician is advisable in the planning stages. Such exercises may collect information on morbidity, mortality, prevalence, incidence and abundance of disease agents or of any other parameter.

Correct and accurate diagnosis is essential when assessing the efficacy of a disease control program and, whenever possible, surveys should contain a rigid diagnostic protocol supported by reliable laboratory tests. This is particularly important for diseases that are difficult to diagnose with certainty. If more than one agent produces a similar clinical disease (e.g., three different viruses can cause similar hemorrhagic disease in deer), the effects of management directed at one of the agents may be masked if the other agents are also present. Although there might be satisfactory control of one causative factor, there might be little obvious impact on the overall disease picture.

The level or degree of sampling depends upon the stage and type of management being done. Initially, when the disease is common, sampling may be quite simple, with the intent of providing a general understanding of where the disease is occurring, a rough indication as to whether the management is having an impact (e.g., changing the prevalence), and to identify problem areas that require more input. If there appears to be an increase in disease in an area, a special investigation may be necessary. As the program proceeds and cases become uncommon, surveillance needs to become more intense and it becomes critical to understand more about the individual cases to assess why disease is still occurring. Laboratory confirmation of diagnosis also becomes increasingly important. For instance in a vaccination program, it would be important to know whether these cases are animals that were in an area where vaccine was placed but which did not contact a bait, or if the animals received bait but were not protected. The former situation might be remedied by more intense distribution of baits, but the latter might indicate a problem with the vaccine.

17.3 The method of assessment

The only way in which the effectiveness of most management programs can be assessed is through some form of planned comparison. The exceptions are programs to prevent the introduction of exotic diseases into an area and during the final stages of a disease eradication program. In these, the effectiveness of the program is judged against an absolute standard, i.e., the continued absence of the disease or its total extirpation, respectively. The problem in these instances is to find a method of detection that is sufficiently sensitive to detect the presence of a very low level of disease. The minimum sample size required to detect disease at various levels of prevalence has been discussed earlier and will not be addressed again here. In all other instances, efficacy is determined through comparison and, wherever possible, the method should involve objective and quantitative, rather than subjective and qualitative comparisons. The object of the comparison is to generate convincing evidence that changes that may have occurred in disease pattern, coincident with the management, were a result of the procedure and were unlikely to have

occurred spontaneously. It is critical to understand the ability (statistical power) of the method used to detect change as a result of the management. A study by Harding et al. (2005), while not related to disease, serves as an example of the use of power analysis to determine the ability to detect changes between years in a wild population. Interannual variation in the number of horned puffins counted at colonies was used to estimate the power to detect changes across years and to choose the most suitable method of sampling. The paper also illustrates the intensity of sampling that is required to be able to detect changes as large as a 60% decrease in population size.

Planned experiments provide the strongest evidence for testing any hypothesis (i.e., H_0 = the treatment is not effective) but these usually are restricted to the planning and preliminary stages of a disease control campaign. However, the experimental method can be applied in many situations through the use of field trials. There are many similarities between laboratory experiments and field trials but there also may be important differences. In laboratory experiments one is able to choose animals for inclusion in the experimental and control groups, and to manipulate both the treatment and the challenge in terms of timing, route of delivery and dose. In field trials, one may be able to choose the groups and control the timing and delivery of the treatment but the challenge is a natural phenomenon and, hence, beyond control. For example, Brown et al. (1990) tried to assess the efficacy of rabies vaccination by comparing survival of radio-collared vaccinated and unvaccinated raccoons in a rabies enzootic area. In this study, no mortality as a result of rabies occurred among either the vaccinated or the unvaccinated groups, so that no conclusion could be drawn about the effectiveness of the vaccine. In a similar study design, Evelsizer (2002) placed radio transmitters on molting mallards on wetlands where botulism was known to occur. The management technique being evaluated was carcass collection. The investigator had no control over which birds were actually exposed to botulinum toxin, but the 30-day survival rate of the marked population on wetlands with and without carcass collection could be compared.

In some disease-management programs, one may not be able to choose the animals for inclusion into the groups or to control either the treatment or the challenge. For example, when baits containing rabies vaccine are placed in the field, it is not possible to control which fox will or will not eat a bait, or when the fox will eat the bait, or if and how the fox will be exposed to rabies virus. Despite these differences, one should strive to choose groups for comparison as carefully in the assessment of disease control operations as is done in laboratory experiments. The methods for choosing suitable controls, for determining appropriate sample sizes, and for choosing statistical tests to analyze the data are similar to those for experiments and were discussed earlier and will not be dealt with here.

There are only three basic ways of making comparisons: (i) comparison of the situation before and after implementation of the management procedure, (ii) comparison between managed and unmanaged groups or areas, and

(iii) a combination of (i) and (ii). It is important to realize that evidence from comparisons between areas and/or comparisons before and after management is, at best, circumstantial. The use of these types of comparison can probably be best illustrated through an example. Assume that the objective of a program is to reduce the risk to humans in an area of enzootic plague, and that the management method used is insecticide treatment of rodent burrows to control fleas. It would be very difficult, in this situation, to measure the risk to humans directly or to detect changes in risk, but one might measure other parameters that relate to human risk, such as the proportion of rodents infected with fleas, the number of fleas/rodent, the proportion of fleas infected with *Yersinia pestis*, and the proportion of rodents with antibodies to *Y. pestis* (the latter is an indicator of plague activity and inter-host transmission of *Y. pestis* in the area).

Obviously, a single measurement of any of these parameters is meaningless without some reference point for comparison. If there was an average of four fleas/rodent after completion of the treatment, this information is of limited value unless it could be compared to the number of fleas prior to treatment, or to the number of fleas present on rodents in untreated areas. The simplest method of comparison would be to collect data by trapping rodents in the area before and after treatment, and then compare the values:

	1 day before treatment	1 week after treatment
Fleas/rodent (mean ± SD)	16 ± 4.2	0.1 ± 0.1
Fleas positive for *Y. pestis* (%)	3	1
Rodents with antibodies (%)	12	12

It appears that there was a decline in the number of fleas and in the proportion of fleas carrying the bacterium at about the time of the treatment. However, this does not prove a cause-effect relationship. The change might have resulted from some other coincidental variation, such as a seasonal change. **Use of pretreatment data as a baseline for comparison assumes that disease parameters continue at a constant rate** and that any change is the result of the management. This is a dangerous assumption in many cases, as most diseases (and biological variables in general) undergo variation in intensity over time. The time span between the two collections was too short in this example for there to have been any change in the prevalence of antibodies, even if the treatment had been effective. Moore et al. (1998) used a before-and-after comparison to measure the effectiveness of a ban on the use of lead shot in preventing ingestion of lead shot by ducks on a lake in Louisiana. The prevalence of shot (lead and steel shot combined) in the gizzards of ducks was similar before and 5 years after the prohibition on use of lead shot, but the proportion of birds with lead shot declined from 27 to 6%.

Another way of assessing the effect of the treatment would be to treat rodents in one or more areas and to choose suitable control areas that were not treated and compare the parameters between the areas. The difficulty in this type of comparison lies in finding areas that are similar in all relevant aspects to the treatment area. Even if the areas are similar at the outset of the management procedure, there is no guarantee that they will remain so during the course of the program, and the evaluator must be constantly vigilant to detect differences. Assuming that suitable control areas were found:

	Treated	Untreated
Fleas/rodent (mean ± SD)	0.1±0.1	4.2±1.3
Fleas positive for *Y. pestis* (%)	1	2
Rodents with antibodies (%)	12	10

Using this method of comparison, there were fewer fleas and a lower proportion of these had *Y. pestis* on the treated area than on the untreated areas but the effect might be attributable to unrecognized differences between the areas, rather than to treatment.

If the parameters are measured on both treated and untreated areas prior to and after treatment, it is possible to evaluate the degree of change associated with the treatment more confidently:

	Treated		Untreated	
	Before	After	Before	After
Fleas/rodent (mean ± SD)	16±4.2	0.1±0.1	12±3.5	4.2±1.2
Fleas positive for *Y. pestis* (%)	3	1	3.4	2
Rodents with antibodies (%)	12	12	13	10

Even without a statistical evaluation, it appears that there was a seasonal decline in the number of fleas on all areas but that the extent of the decline was more marked in the treated area than in the untreated area. If sufficient data were available, the proportion of the decline attributable to treatment could be calculated. It appears that the treatment had little effect on the proportion of fleas carrying the bacterium or on the prevalence of antibodies in the rodents. Cavanaugh et al. (1972) described a situation similar to this hypothetical example in which serologic tests of rodent sera clearly demonstrated that a massive control program for rodent plague in Vietnam was ineffective. This led to the discovery that the flea vector had become resistant to the insecticide being used.

Monitoring of the effectiveness of a management program must be planned in advance of starting a project, so that pre-treatment samples can be collected and so that suitable control areas can be selected. The number

and type of parameters that can be compared in various ways is limited only by the imagination of the investigator and his or her budget.

17.4 The time frame for assessing effectiveness

The assessment process should begin before any disease-management measures are taken and evaluation should occur, at least periodically and preferably continuously, during the course of the program. This is done to ensure that if a technique is ineffective, this fact will be detected early in the program, and so that timely improvements and corrections can be made. Assessment of current management outcomes so that future effectiveness can be improved is the basis of adaptive management (Holling 1978). Ongoing assessment is needed because a technique that is effective early in a campaign may lose its effectiveness with use. Diseases, particularly those caused by infectious agents, refuse to present a stationary target that can be hit repeatedly by the same technique over an extended period of time. Experience in control of human and livestock diseases indicates that problems, such as the development of antibiotic, pesticide or toxin resistance among agents, hosts or vectors, genetic diversity among agents, and changes in environmental conditions, often lead to the eventual failure of methods that were once effective.

It is very important to relate the timing of the assessment procedure to the objectives and the expected duration of effects of the disease-management program. For instance, if the objective of a program is an immediate effect, such as might be expected after a single application of pesticide to control an arthropod vector, it would be appropriate to collect samples the day before, and a few days after the pesticide application. If the desired effect is a long-term reduction in the prevalence of a disease, sampling may have to be continued for months or years to assess the effect, particularly when dealing with chronic disease in a long-lived species. Different parameters may have to be measured at different times for the assessment of a single operation. If we return to the example of using insecticide to reduce the risk of sylvatic plague, one would expect that the initial efficacy of the technique in killing fleas could be measured within hours or days of the treatment. However, it is important to measure over a longer period to determine how rapidly the flea population rebuilds. The prevalence of antibodies among rodents would not change appreciably for weeks, even if the treatment had been successful in reducing transmission of the agent and the incidence rate of infection among the rodents. In this case, although the time frame for the two parameters is different, one should probably sample both the prevalence of fleas and measure the occurrence of antibody over a period of weeks following the pesticide application to monitor the efficacy and the duration of the treatment program. In the case of infectious conditions, the assessment program must be continued for at least as long as the maximum incubation period known for the disease. Thus, if one were trying to assess the efficacy of a program to

eradicate tuberculosis, the monitoring would have to continue for several years to assure that all cases were eliminated. A study of the effectiveness of a DNA vaccine in protecting canaries against avian malaria illustrates the need for assessing both short-term and long-term effects of management (McCutchan et al. 2004). In the year of vaccination immunized birds had significantly reduced mortality compared to non-immunized birds. However, in the following season, the mortality rate among birds immunized in year 1 was significantly greater than that of non-immunized birds that had survived infection the preceding year. The conclusion was that vaccine-induced immunity prevented acquisition of protective natural immunity.

It is usually much easier to assess the short-term effect of a disease control program than to monitor its long-term efficacy. In designing an assessment plan it is useful to consider which of these is of greater biological significance. When waterfowl are treated with antitoxin for avian botulism, or seabirds are rehabilitated during oil spills, the success of the treatment usually has been assessed by the proportion of birds that survive treatment and fly away. No attempt is made to monitor the long-term survival of these recovered birds, so it is unclear whether or not the management activities actually contribute to the reproductive potential of the species. This type of long-term assessment could be done through the analysis of band-returns and might provide a more meaningful measure of the true efficacy of the programs (incidentally, the survival rate among birds treated for botulism has never been compared to that of similarly affected birds left in the marsh without treatment, so that even the short-term efficacy of this treatment is unproven). Similar comments could be made about many programs of disease management that are based on reduction of the number or density of a host population. The number of animals removed from the population during any year is probably not a good indicator of the efficacy of the program and one should look carefully at the duration of the effect and its impact on the transmission and prevalence of the disease over an extended period to measure the impact of the management.

17.5 Assessing the economics of disease management

The financial aspects of disease prevention and control have received considerable attention in human and veterinary medicine. The aims of this type of assessment are to obtain the maximum effect for dollars spent in health care and to evaluate the economic benefits of action as compared to the costs of inaction. In human medicine, economic benefits can be measured in quantifiable terms such as savings in treatment cost through disease prevention, increased productivity through reduced absenteeism, and lost wages as a result of disease. The benefits of disease management in domestic animals may be measured in terms such as increased rate of gain, increased egg production, higher calving percentage, lower treatment costs, or reduced

culling. The economics of wildlife disease (and of wild animals in general) are much more difficult to define. For example, as noted earlier, no one has attempted to analyze the value of a duck 'saved' from botulism. In some situations, particularly with game species, it may be possible to calculate a financial benefit from preventing or reducing disease. A trophy bighorn ram has a certain definable value based on economic returns from license sales, guiding, lodging, feeding, and supplying the hunters that pursue the animal. This value could be calculated and compared to the costs involved in preventing the occurrence of fatal pneumonia in the group of sheep to which the ram belongs. Similarly, the increased production of young that resulted from treatment of red grouse for *Trichostrongylus tenuis* (Hudson 1986) could be given a value in terms of the increased financial return from hunting on the treated area. In many other instances, it would be difficult or inappropriate to evaluate the effects of disease in financial terms. For instance, the financial loss when a mute swan dies of lead poisoning on the River Thames or when a brown pelican population fails to reproduce as a result of pesticide contamination have never been calculated. In such instances, non-financial considerations are much more important than any financial value placed on the birds. The decision as to whether the benefit of disease management is sufficient to justify the expenditure must be based on ethical and political considerations, rather than being based on economics.

Despite the difficulty in placing values on wild animals, economics will play a role in any disease-management program and the evaluation of a program should contain some form of economic analysis. This may take the form of either **benefit:cost** analysis or **cost:effectiveness** analysis. Benefit:cost analysis is used when both the benefits and the costs of an action can be quantified with a common denominator, usually their monetary value. The basic principle of benefit:cost analysis is one of balancing, so that action is taken only when benefits outweigh costs. The costs of an action are usually much easier to measure than are the benefits. One can measure the manpower, vehicle mileage and consumables used in a management program directly and assign a dollar value to these costs. However, in order to assess the benefits, one has to know the effect and the cost of the disease in the absence of any management, as well as the change in the level of disease that occurs as a result of the procedure. This process can become very complex because most diseases do not occur at a uniform rate. This is especially problematic for diseases that occur sporadically. For example, botulism might occur in a marsh during 2 years in a decade and the number of birds involved in any one year may be highly variable. In such circumstances, the calculations must include some consideration of risk. Another factor that introduces complexity into the calculations is that the costs for the management program may be separated in time from the benefits. If habitat modifications were done to reduce the effects of botulism in the hypothetical marsh described above, there might be no detectable benefit until several years after the work is completed and paid for. Techniques are available for adjusting

economic values to account for the time that they occur and these are used commonly in benefit:cost analyses. Non-financial consequences of the action also may be included in the tabulation of benefits and costs and used in the final decision making process.

Cost:effectiveness analysis does not require placing a financial value on the benefits of disease control; only the costs are considered. This type of evaluation is probably more widely applicable to disease situations in wild animals than is benefit:cost analysis. Cost:effectiveness analysis may be used in several ways including situations where: (i) the benefit is difficult to quantify in financial terms (e.g., the reduction in risk of human infection with plague from wild rodents or rabies from skunks), (ii) the action is deemed to be necessary for non-financial reasons (e.g., reduction of contamination of raptors with persistent insecticides), and (iii) several different alternative actions might result in the same effect. In such situations, it is assumed that some management action will take place. The purpose of the evaluation is to determine how a desired result can be achieved at minimum cost or which among a group of alternative actions will provide the greatest benefit for a fixed investment (the biggest bang for the buck!). As an example of this type of analysis, one might decide that rabies must be controlled in an area and that the disease could be eliminated from a population of foxes either by reducing the population density of foxes to $<0.2/km^2$ and maintaining the population at that level through gassing of dens and poisoning **or** by immunizing a minimum of 60% of the fox population. The result of the two actions, in terms of disease control, might be the same but the costs might be substantially different. In this example, there also might be benefits from one or the other action that cannot be quantified. For instance, immunization would likely be more favorably received by the public than would wide-scale poisoning. This could be of great political value in having the project funded, although impossible to express in financial terms.

17.6 Using models to predict and assess effectiveness of programs

Evaluation of the effectiveness of disease-management programs is a difficult and expensive process, and comparison of the relative merits of several different techniques under field conditions is beyond the capability of most agencies. An alternative to field tests is the use of mathematical simulations or models. Almost any biological process can be represented by a mathematical model and the model may be simple or very complex, depending upon the amount of quantitative information that is available on the many factors that influence disease. The great advantage of models is that various factors can be manipulated and alternative techniques can be tested easily and rapidly.

A further advantage is that one can learn through trial and error, without having to pay a heavy price for damage caused by errors.

Probably the most common use of models in disease-management programs is in the planning stage, where the model can be used in determining the feasibility of management, for choosing the most appropriate method from among a number of alternatives, and for establishing realistic objectives and goals. For instance, Bacon (1981) used a model to predict the probable spread of rabies after introduction into Great Britain. The model suggested that by the time the disease was discovered it was likely that it would already have spread over such a large area that eradication by depopulation would no longer be feasible. The design of models is beyond the scope of this book but a number of examples are available of the use of models for these purposes. Habtemariam et al. (1983) used a model to evaluate the relative efficiency of different techniques for controlling trypanosomiasis in African cattle. These authors began by using the current prevalence of the disease in the area as the baseline for comparison, and then introduced various control measures singly, or in combination, into the model and followed the disease prevalence for "*10 years*". Results from this model indicated that an integrated program including several different procedures was the most effective and feasible method for disease management. Other examples of the use of modeling to assess the relative effectiveness of different management strategies include Barlow (1991a, 1991b, 1993), Swinton et al. (1997), Hayes et al. (1999), Gormly and Collins (2000), Selhorst et al. (2001), and Smith and Wilkinson (2003).

An important attribute of many models is that they provide a quantitative estimate of the degree of coverage that is required for a method, such as immunization or population reduction, to be effective. This estimate can be used in establishing objectives and as a standard against which the actual method can be measured in the field. For example, various models have predicted that 50–65% of foxes in a population (depending on the population density) must be immunized for eradication of rabies (Berger 1976; Bacon and MacDonald 1980; Anderson et al. 1981; Artois et al. 1997). Steck et al. (1982) found that vaccination at about this level did, in fact, result in effective control of rabies in Switzerland. Because models use a mathematical base, it is relatively easy to include economic factors into the equation and, hence, to do an assessment of both the biological and the economic effectiveness of a program at one time.

17.7 Summary

- The effectiveness of most procedures used for management of diseases in wild animals is untested and unknown.
- Every disease-management program should include techniques to measure its effectiveness.

- Methods for assessing the efficacy of a program must be planned before the program is commenced.
- Assessment usually involves a comparison among treated and untreated populations or areas. General rules for choosing appropriate controls and samples apply.
- The parameters used for measuring success must be directly relevant to the objectives of the program.
- Different parameters may have to be used for evaluating effectiveness at various stages of a program.
- Data collection and analysis should begin before any management is done so that ineffective methods can be detected early in the program and corrected.
- Assessment should continue throughout the program so that changes in the effectiveness of methods can be detected.
- Methods for analyzing the relative benefit:cost and cost:effectiveness of procedures are available and should be applied.
- Mathematical models may be very useful in assessing the efficacy of techniques.

Common and scientific names of animals

American avocet *Recurvirostra americana*
Antelope, roan *Hippotragus equinus*
Auklet, Cassin's *Ptychoramphus aleuticus*
Baboon, Chacma *Papio ursinus*
Badger *Meles meles*
Bat, big brown *Eptesicus fuscus*
 vampire *Desmodus rotundus*
Bear,
 black *Ursus americanus*
 Grizzly *Ursus arctos*
Beaver *Castor canadensis*
Bison *Bison bison*
 Wood *Bison b. athabascae*
Bobcat *Lynx rufus*
Bobwhite, northern *Colinus virginianus*
Bongo *Tragelaphus eurycerus*
Brant, Atlantic *Branta bernicla*
Buffalo,
 African *Syncerus caffer*
 water *Bubalus bubalus*
Bullfrog *Rana catesbeiana*
Capercaillie *Tetrao urogallus*
Caribou,
 barren-ground *Rangifer tarandus groenlandicus*
 woodland *Rangifer tarandus caribou*
Chamois *Rupicapra rupicapra*
Coot, American *Fulica americana*
Cormorant,
 double-crested *Phalacrocorax auritus*
 olivaceous *Phalacrocorax olivaceus*
Coyote *Canis latrans*
Chough, red-billed *Pyrrhocorax pyrrhocorax*
Coypu *Myocastor coypus*
Crab, horseshoe *Limulus polyphemus*
Crane,
 sandhill *Grus canadensis*
 whooping *Grus americana*
Crow, common *Corvus brachyrhynchos*

Deer,
Andean *Hippocamelus bisulcus*
black-tailed *Odocoileus hemionus columbianus*
fallow *Dama dama*
maral *Cervus elaphus*
mule *Odocoileus h. hemionus*
red *Cervus elaphus*
roe *Capreolus capreolus*
sika *Cervus nippon*
white-tailed *Odocoileus virginianus*
Dove, white-winged *Zenaida asiatica*
Duck, black *Anas rubripes*
Eagle,
bald *Haliaeetus leucocephalus*
golden *Aquila chrysaetos*
Eider, common *Somateria mollissima*
Elk (wapiti) *Cervus elaphus*
Falcon, peregrine *Falco peregrinus*
Ferret, *Mustela furo*
black-footed *Mustela nigripes*
Finch, house *Carpodacus mexicanus*
Fox,
arctic *Alopex lagopus*
gray *Urocyon cinereoargenteus*
red *Vulpes vulpes*
Gannet *Sula bassanus*
Gerbil, great *Rhombomys opimus*
Goose,
Canada *Branta Canadensis*
Emperor *Chen canagica*
greater snow *Chen caerulescens atlantica*
greylag *Anser anser*
lesser snow *Chen c. caerulescens*
pink-footed *Anser brachyrhynchus*
Ross' *Chen rossii*
white-fronted *Anser albifrons*
Grebe, eared *Podiceps nigricollis*
Ground squirrel,
Franklin's *Spermophilus franklinii*
Richardson's *Spermophilus richardsonii*
Grouse,
black *Tetrao tetrix*
red *Lagopus lagopus scoticus*
sage *Centrocercus urophasianus*
sharp-tailed *Pediocetes phasianellus*
willow *Lagopus lagopus*
Guillemot *Uria* spp.
Gull,
California *Larus californicus*

Franklin's *Larus pipixcan*
glaucous *Larus hyperboreus*
herring *Larus argentatus*
Gypsy moth *Lymantria dispar*
Hare,
European *Lepus europaeus*
mountain *Lepus timidus*
snowshoe *Lepus americana*
Harrier *Circus cyaneus*
Hawk,
Cooper's *Accipiter cooperii*
red-tailed *Buteo jamaicensis*
Heron, great blue *Ardea herodias*
Ibex, Spanish *Capra pyrenaica*
Impala *Aepyceros melampus*
Jackal *Canis* spp.
Jay, blue *Cyanocitta cristata*
Junco, dark-eyed *Junco hyemalis*
Kakapo *Strigops habroptilus*
Kestrel *Falco tinnunculus*
Knot, red *Calidris canutus rufa*
Koala *Phascolarctos cinereus*
Kudu *Tragelaphus strepsiceros*
Lion *Panthera leo*
Llama *Llama glama*
Loon, common *Gavia immer*
Lynx *Lynx lynx*
Magpie, black-billed *Pica pica*
Mallard *Anas platyrhynchos*
Mongoose *Herpestes auropunctatus*
Monkey, squirrel *Saimiri sciureus*
Moose *Alces alces*
Mouse, house *Mus musculus*
deer *Peromyscus maniculatus*
white-footed *Peromyscus leucopus*
wood *Apodemus sylvaticus*
yellow-necked *Apodemus flavicollis*
Muskrat *Ondatra zibethica*
Northern goshawk *Accipiter gentiles*
Northern Pintail *Anas acuta*
Oryx, Arabian *Oryx leucoryx*
Osprey *Pandion haliaetus*
Otter, river *Lutra canadensis*
Panda, lesser *Ailurus fulgens*
Partridge,
grey *Perdix perdix*
red-legged *Alectoris rufa*
Pelican, brown *Pelecanus occidentalis*
Penguin, African black-footed *Spheniscus demersus*

Pheasant, ring-necked *Phasianus colchicus*
Pig, feral *Sus scrofa*
Pigeon,
    domestic *Columba livia*
    wood *Columbo p. palumbo*
Polecat, Siberian *Mustela eversmanni*
Possum, brushtail *Trichosurus vulpecula*
Prairie chicken, Attwater's *Tympanuchus cupido attwateri*
Prairie dog, black-tailed *Cynomys ludovicianus*
Pronghorn *Antilocapra americana*
Ptarmigan *Lagopus mutus*
    Willow *Lagopus lagopus*
Puffin, horned *Fratercula corniculata*
Rabbit,
    cottontail *Sylvilagus floridanus*
    European *Oryctolagus cuniculus*
Raccoon *Procyon lotor*
Raccoon dog *Nyctereutes procyonoides*
Rat,
    bush *Rattus fuscipes*
    cotton *Sigmodon hispidus*
Reindeer *Rangifer tarandus tarandus*
Robin *Turdus migratorius*
Rook *Corvus frugilegus*
Sandpiper, pectoral *Calidris melanotos*
Scaup, lesser *Aythya affinis*
Seal, harbour *Phoca vitulina*
Shrike, Eastern loggerhead *Lanius ludovicianus migrans*
Skunk, striped *Mephitis mephitis*
Sheep,
    Dall *Ovis dalli*
    bighorn *Ovis canadensis*
    Soay *Ovis aries*
Sparrow,
    chipping *Spizella passerina*
    house *Passer domesticus*
Sparrowhawk *Accipiter nisus*
Squirrel,
    red *Sciurus vulgaris*
    grey *Sciurus carolinensis*
    rock *Spermophilus variegates*
Starling *Sturnus vulgaris*
Stilt, black-necked *Himantopus mexicanus*
Swallow,
    barn *Hirundo rustica*
    cliff *Hirundo pyrrhonota*
Swan,
    mute *Cygnus olor*
    tundra *Olor columbianus*
    whooper *Cygnus cygnus*

Teal, blue-winged *Anas discors*
Tern, black *Chlidonias niger*
Thrasher, sage *Oreoscoptes montanus*
Turkey, wild *Meleagridis gallopava*
Vole,
    bank *Clethrionomys glareolus*
    Brandt's *Microtus brandti*
    common *Microtus arvalis*
    meadow *Microtus pennsylvanicus*
    sibling *Microtus rossiaemeridionalis*
    water *Arvicola terrestris*
Vulture, white-tailed griffon *Gyps africanus*
Warthog *Phacochoerus africanus*
Weasel, long-tailed *Mustela frenata*
Wildebeest *Connochaetes taurinus*
Wolf *Canis lupus*
Wolf, Ethiopian *Canis simensis*
Woodrat, Allegheny *Neotoma magister*

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